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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

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1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Wallette™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.

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ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.
MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy. Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease.* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences,* or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned.
Precautions *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings.
Adverse Reactions An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment); gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

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An established standard of efficacy

in serious pelvic infection

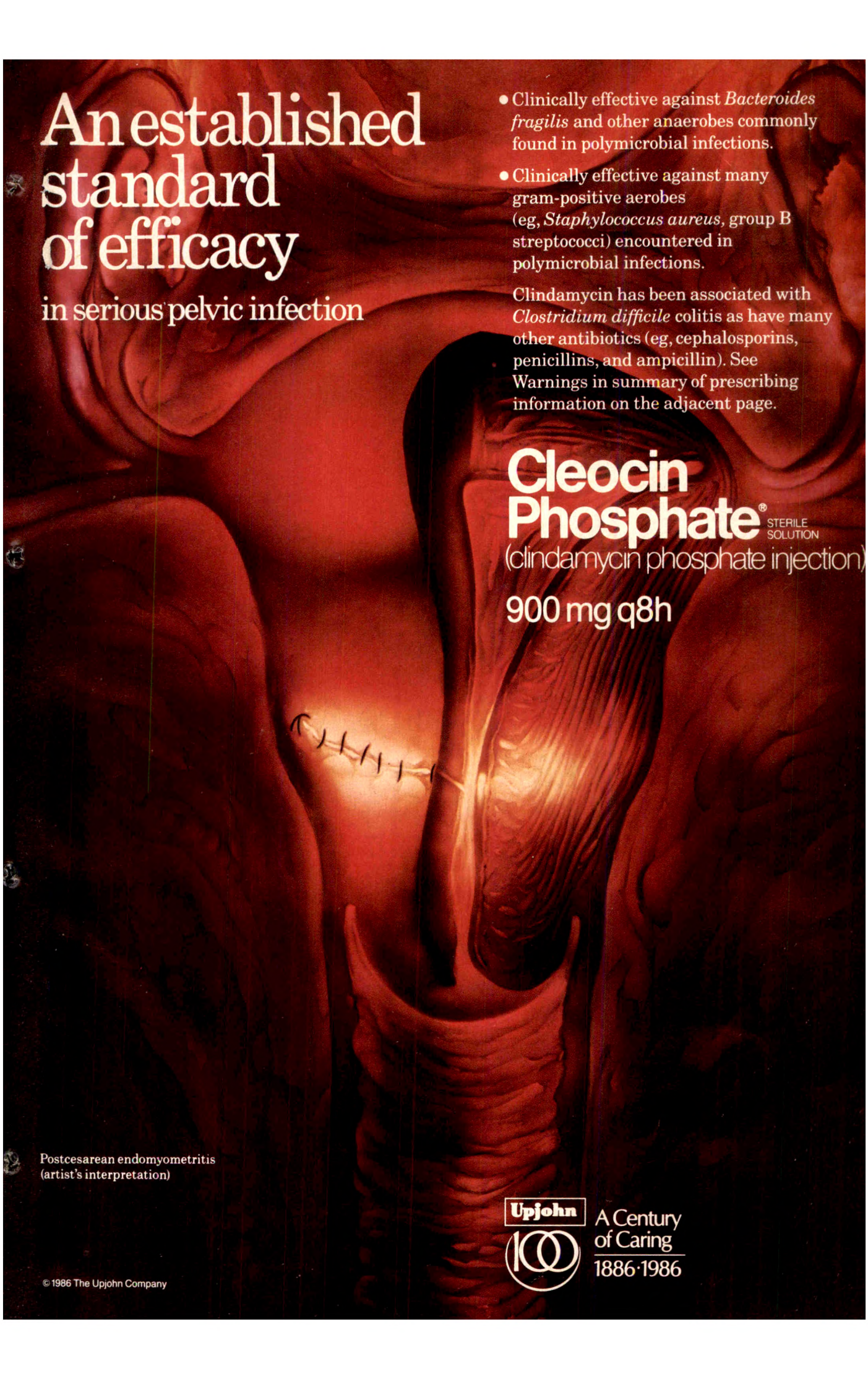
- Clinically effective against *Bacteroides fragilis* and other anaerobes commonly found in polymicrobial infections.
- Clinically effective against many gram-positive aerobes (eg, *Staphylococcus aureus*, group B streptococci) encountered in polymicrobial infections.

Clindamycin has been associated with *Clostridium difficile* colitis as have many other antibiotics (eg, cephalosporins, penicillins, and ampicillin). See Warnings in summary of prescribing information on the adjacent page.

Cleocin Phosphate® STERILE SOLUTION

(clindamycin phosphate injection)

900 mg q8h



Postcesarean endomyometritis (artist's interpretation)

Upjohn 100 A Century of Caring 1886·1986

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CLEOCIN PHOSPHATE® Sterile Solution
CLEOCIN HCl® Capsules
(clindamycin)

WARNING

Clindamycin therapy has been associated with severe colitis which may end fatally. Therefore, it should be reserved for serious infections where less toxic antimicrobial agents are inappropriate, as described in the Indications Section. It should not be used in patients with nonbacterial infections, such as most upper respiratory tract infections. Studies indicate a toxin(s) produced by Clostridia is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. See WARNINGS section. The colitis is usually characterized by severe, persistent diarrhea and severe abdominal cramps and may be associated with the passage of blood and mucus. Endoscopic examination may reveal pseudomembranous colitis.

When significant diarrhea occurs, the drug should be discontinued or, if necessary, continued only with close observation of the patient. Large bowel endoscopy has been recommended.

Antiperistaltic agents such as opiates and diphenoxylate with atropine (Lomotil) may prolong and/or worsen the condition. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dose is 500 milligrams to 2 grams of vancomycin orally per day in three to four divided doses administered for 7 to 10 days. Cholestyramine or colestipol resins bind vancomycin *in vitro*. If both a resin and vancomycin are to be administered concurrently, it may be advisable to separate the time of administration of each drug.

Diarrhea, colitis, and pseudomembranous colitis have been observed to begin up to several weeks following cessation of therapy with clindamycin.

INDICATIONS

Serious infections caused by susceptible anaerobic bacteria. Patients with serious infections due to susceptible strains of streptococci, pneumococci, and staphylococci in whom its use should be reserved for penicillin-allergic patients or other patients for whom, in the judgment of the physician, a penicillin is inappropriate.

Consider the nature of the infection and the suitability of less toxic alternatives (e.g., erythromycin). Bacteriologic studies should be performed to determine the causative organisms and their susceptibility to clindamycin.

CONTRAINDICATIONS

History of hypersensitivity to clindamycin or lincomycin.

WARNINGS

See WARNING box. A toxin produced by Clostridia is one primary cause of antibiotic associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed promptly with fluid, electrolyte and protein supplementation as indicated. Vancomycin has been found to be effective in the treatment of antibiotic associated pseudomembranous colitis produced by *Clostridium difficile*. The usual adult dosage is 500 mg to 2 grams of vancomycin orally per day in 3 or 4 divided doses for 7 to 10 days. Systemic corticoids and corticoid retention enemas may help relieve the colitis. Other causes of colitis should also be considered.

A careful inquiry should be made concerning previous sensitivities to drugs and other allergens. Because antagonism has been demonstrated between clindamycin and erythromycin *in vitro*, these drugs should not be administered concurrently. *Usage in Pregnancy:* Safety has not been established. *Usage in Newborns and Infants:* Appropriate monitoring of organ system functions is desirable. *Nursing Mothers:* Clindamycin has been reported to appear in breast milk in ranges of 0.7 to 3.8 mcg/ml. *Usage in Meningitis:* Since clindamycin does not diffuse adequately into the cerebrospinal fluid, it should not be used to treat meningitis.

SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN AND INTRAVENOUS CORTICOSTEROIDS SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS

Older patients with associated severe illness may tolerate diarrhea less well. When clindamycin is indicated in these patients, they should be carefully monitored for change in bowel frequency. Prescribe with caution in individuals with a history of gastrointestinal disease, particularly colitis and also in atopic individuals. Indicated surgical procedures should be performed in conjunction with therapy. Patients with severe renal disease and/or very severe hepatic disease accompanied by severe metabolic aberrations should be dosed with caution and serum clindamycin levels monitored during high dose therapy.

During prolonged therapy, periodic liver and kidney function tests and blood counts should be performed. Use may result in overgrowth of nonsusceptible organisms, particularly yeasts. Clindamycin has neuromuscular blocking properties and may enhance other neuromuscular blocking agents. Use with caution in patients receiving such agents. Do not inject clindamycin IV undiluted as a bolus. Dilute prior to IV administration to 300 mg per 50 ml or more of diluent. Infuse over at least 10-60 minutes. (See Dosage and Administration.) CLEOCIN HCl Capsules contain FD&C Yellow No. 5 (tartrazine) which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, especially in patients who also have aspirin hypersensitivity.

ADVERSE REACTIONS

Gastrointestinal: Abdominal pain, nausea, vomiting and diarrhea. (See WARNING box.)

Hypersensitivity Reactions: Maculopapular rash and urticaria. Generalized mild to moderate morbilliform-like skin rashes are the most frequent adverse reactions. Rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been reported. A few cases of anaphylactoid reactions have been reported. If a hypersensitivity reaction occurs, the drug should be discontinued. The usual agents should be available for emergency treatment. *Liver:* Jaundice and abnormalities in liver function tests have been observed. *Hematopoietic:* Neutropenia, eosinophilia, agranulocytosis and thrombocytopenia have been reported; no direct etiologic relationship to concurrent clindamycin therapy has been made. *Local Reactions:* Pain, induration and sterile abscess have been reported after intramuscular injection and thrombophlebitis after intravenous infusion. Reactions can be minimized or avoided by giving deep intramuscular injections and avoiding prolonged use of indwelling intravenous catheters. *Musculoskeletal:* Rare instances of polyarthritis have been reported. *Cardiovascular:* Rare instances of cardiopulmonary arrest and hypotension have been reported following too rapid IV infusion. (See Dosage and Administration.) *Renal:* Renal dysfunction has rarely been observed. No direct relationship has been established.

HOW SUPPLIED

Available as sterile solution with each ml containing clindamycin phosphate equivalent to 150 mg clindamycin base. Vials of 2 ml, 4 ml, and 6 ml.

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Contents

April
1986

This issue contains Transactions of the Fourth Annual Meeting of the American Gynecological and Obstetrical Society, a Clinical Articles section, beginning on page 855, and a Symposium on Cefotetan, beginning on page 945.

TRANSACTIONS OF THE FOURTH ANNUAL MEETING OF THE AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY

Words, thoughts, and things 699
Joseph Seitchik, M.D.
San Antonio, Texas

Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis 709
Tsutomu Ono, M.D., Joseph D. Campeau, M.S., Eric A. Holmberg, B.A., Robert M. Nakamura, Ph.D., Eryn L. Ujita, B.A., Dawn L. Devereaux, B.A., Sharon A. Tonetta, Ph.D., Robin DeVinna, B.A., Maria Ugalde, M.S., and Gere S. diZerega, M.D.
Los Angeles, California, and Yamagata, Japan

(Contents continued on page 7A)

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Contents continued from page 5A

Further studies on genital mycoplasms in intra-amniotic infection: Blood cultures and serologic response 717
Ronald S. Gibbs, M.D., Gail H. Cassell, Ph.D., Jerry K. Davis, D.V.M., Ph.D., and P. J. St. Clair, B.A.
San Antonio, Texas, and Birmingham, Alabana

The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo 727
Grant E. Schmidt, M.D., Ph.D., Moon H. Kim, M.D., Ragaa Mansour, M.D., Lynne Torello, M.D., and Chad I. Friedman, M.D.
Columbus, Ohio

Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes 737
Paul G. McDonough, M.D., Sandra P. Tho, M.D., John J. Trill, M.S., J. Rogers Byrd, Ph.D., Richard H. Reindollar, M.D., and Jay A. Tischfield, Ph.D.
Augusta, Georgia

Partial purification of relaxin from human seminal plasma 749
Gerson Weiss, M.D., Laura T. Goldsmith, Ph.D., Cy Schoenfeld, Ph.D., and Richard D'Eletto, B.S.
New York, New York

Human ovarian oxytocin: Its source and relationship to steroid hormones 756
M. Yusoff Dawood, M.D., and Firyal S. Khan-Dawood, Ph.D.
Chicago, Illinois

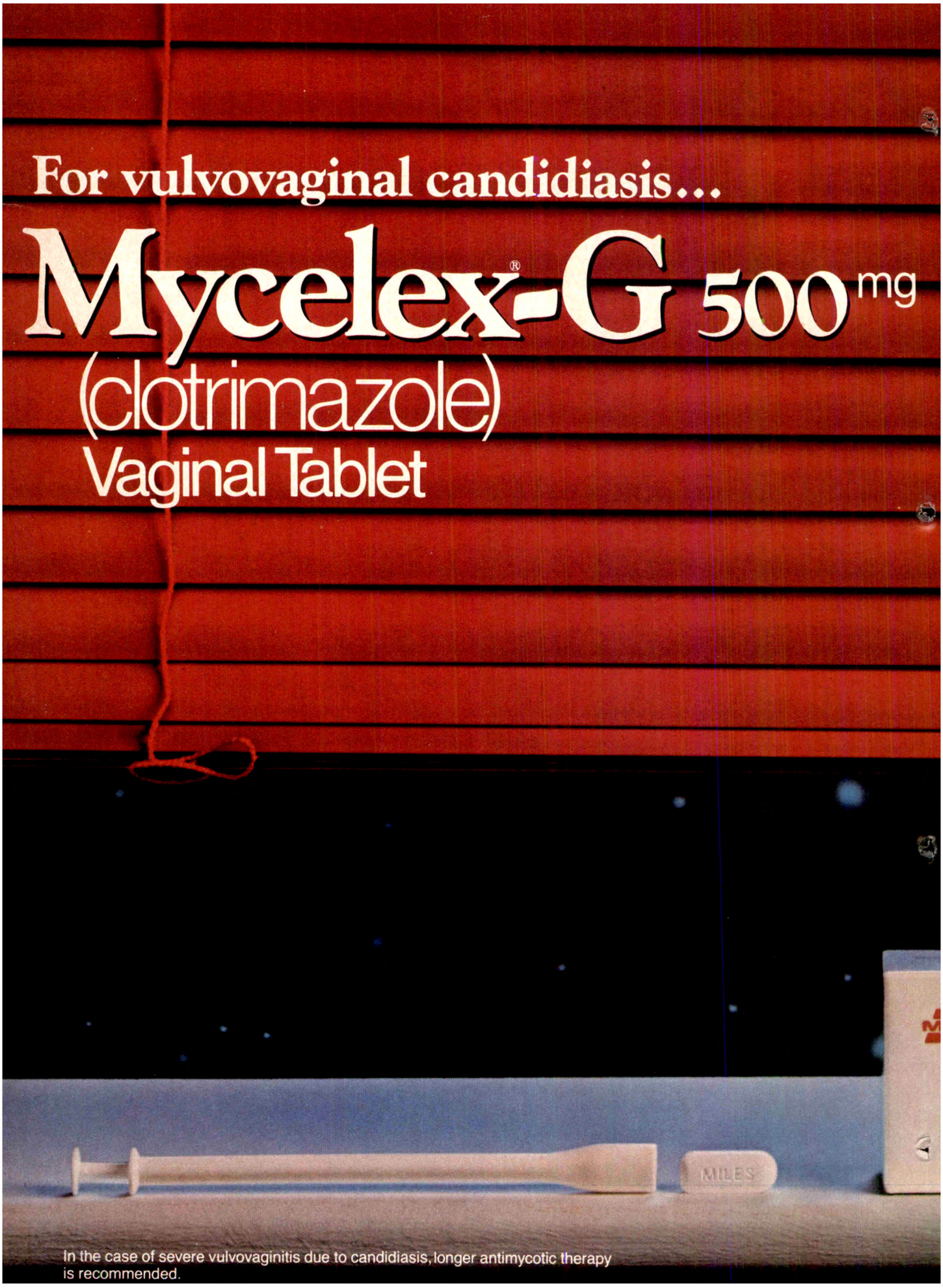
Midforceps deliveries: Long-term outcome of infants 764
LeRoy J. Dierker, Jr., M.D., Mortimer G. Rosen, M.D., Karen Thompson, L.P.N., and Patricia Lynn, Ph.D.
Cleveland, Ohio, and New York, New York

The relationship between antepartum fetal heart rate, intrapartum fetal heart rate, and fetal acid-base status 769
James A. Low, M.D., Michael J. McGrath, M.D., Sharon J. Marshall, R.T., Anneke Fischer-Fay, B.A., and E. Jane Karchmar, B.A.
Kingston, Ontario, Canada

Symposium on the need for flexibility in academic obstetric-gynecologic residencies

Flexibility in obstetric-gynecologic residencies: Why is it necessary? Introduction 777
J. Robert Willson, M.D.
Albuquerque, New Mexico

(Contents continued on page 11A)


For vulvovaginal candidiasis...
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INDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis, *Trichomonas* and *Gardnerella (Haemophilus) vaginalis*, should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

WARNINGS: None.

PRECAUTIONS: If there is a lack of response to Mycelex®-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

CARCINOGENESIS: No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex®-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

USAGE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

ADVERSE REACTIONS: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex®-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSAGE: No data available.

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex®-G 500 mg Vaginal Tablets has not been reported.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

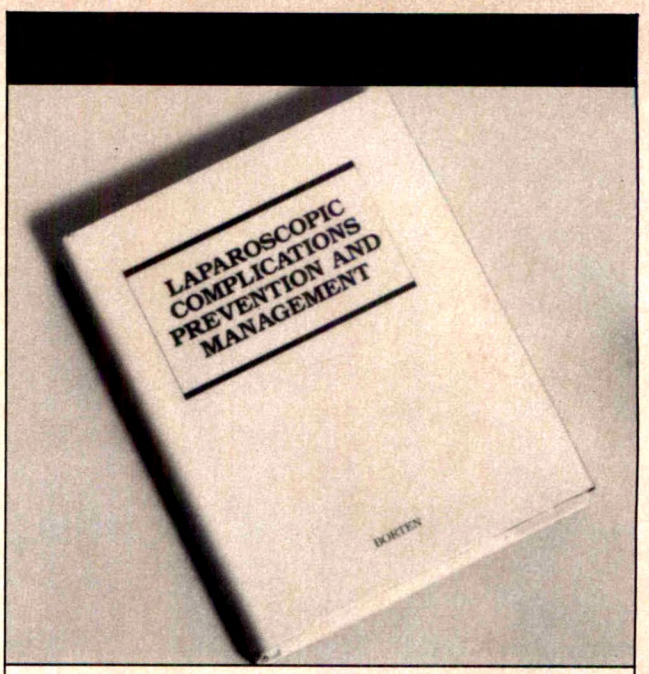
HOW SUPPLIED: Mycelex®-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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Contents continued from page 7A

The need for flexibility in preparing clinician-scientists for academic careers 778
Robert B. Jaffe, M.D.
San Francisco, California

Experience with flexibility in an academic residency program 780
Charles H. Hendricks, M.D.
Chapel Hill, North Carolina

Response on the need for flexibility in academic obstetric-gynecologic residencies 785
James A. Merrill, M.D.
Seattle, Washington

Response on the need for flexibility in academic obstetric-gynecologic residencies 788
Roy M. Pitkin, M.D.
Iowa City, Iowa

The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome 792
John W. Goldkrand, M.D., and Armando M. Fuentes, M.D.
Albany, New York

Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy 801
Mary Ellen Dorko, B.S., and T. Terry Hayashi, M.D.
Pittsburgh, Pennsylvania

Uterine artery Doppler velocimetry in pregnant women with hypertension 806
Adiel Fleischer, M.D., Harold Schulman, M.D., George Farmakides, M.D., Luis Bracero, M.D., Lawrence Grunfeld, M.D., Burton Rochelson, M.D., and Mordecai Koenigsberg, M.D.
Stony Brook, New York

Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study 814
Arthur L. Herbst, M.D., Sharon Anderson, Ph.D., Marian M. Hubby, Ph.D., William M. Haenszel, Dr.P.H., Raymond H. Kaufman, M.D., and Kenneth L. Noller, M.D.
Chicago, Illinois, Houston, Texas, and Rochester, Minnesota

Effect of induced oligohydramnios on fetal lung development 823
Jeffrey C. King, M.D., Wayne Mitzner, Ph.D., Arthur B. Butterfield, D.V.M., Ph.D., and John T. Queenan, M.D.
Washington, D.C., and Baltimore, Maryland

(Contents continued on page 13A)

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Contents continued from page 11A

Temporal and receptor correlates of the estrogen response in sheep 831
Frank C. Greiss, Jr., M.D., James C. Rose, Ph.D., Timothy E. Kute, Ph.D., Randall T. Kelly, M.D., and Lisa S. Winkler, B.S.
Winston-Salem, North Carolina

Histologic and hormonal documentation of the luteinized unruptured follicle syndrome 839
Robert S. Schenken, M.D., Lawrence B. Werlin, M.D., Robert F. Williams, Ph.D., Thomas J. Prihoda, Ph.D., and Gary D. Hodgen, Ph.D.
San Antonio, Texas, and Bethesda, Maryland

An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries 848
Mason C. Andrews, M.D., Suheil J. Muasher, M.D., Donald L. Levy, M.D., Howard W. Jones, Jr., M.D., Jairo E. Garcia, M.D., Zev Rosenwaks, M.D., Georgeanna S. Jones, M.D., and Anibal A. Acosta, M.D.
Norfolk, Virginia

CLINICAL SECTION

Clinical Articles

Focal vulvitis: A characteristic syndrome and cause of dyspareunia 855
Ben M. Peckham, M.D., Ph.D., Dennis G. Maki, M.D., Jeffrey J. Patterson, D.O., and Gholam-Reza Hafez, M.D.
Madison, Wisconsin

Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease 865
Nancy B. Kiviat, M.D., Pål Wølner-Hanssen, M.D., Marcia Peterson, C.T., Judy Wasserheit, M.D., Walter E. Stamm, M.D., David A. Eschenbach, M.D., Jorma Paavonen, M.D., Julie Lingenfelter, C.T., Thomas Bell, M.D., Vinette Zabriskie, M.D., Barbara Kirby, M.D., and King K. Holmes, M.D., Ph.D.
Seattle, Washington

Obstetric hospital stays by diagnosis related groups: A community-based analysis 873
Ronald J. Lagoe, Ph.D.
Syracuse, New York

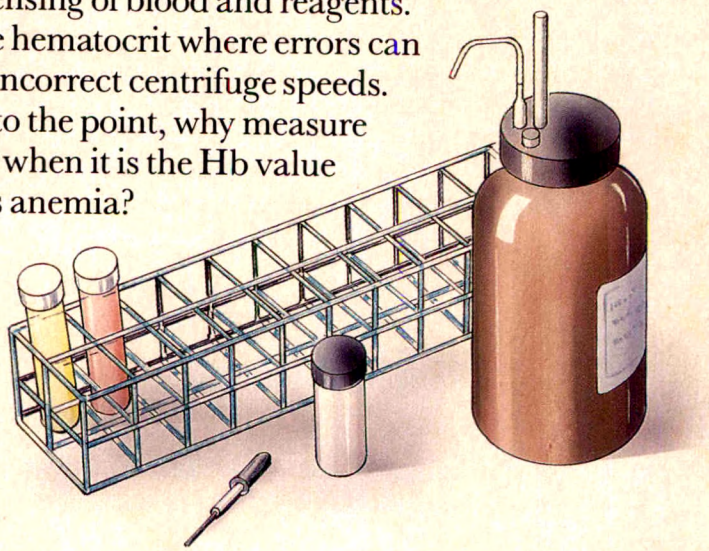
An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody 878
David Hart, M.D., Carol Dunetz, M.D., Michael Nardi, M.S., Robert F. Porges, M.D., Agnes Weiss, M.S., and Margaret Karpatkin, M.D.
New York, New York

(Contents continued on page 16A)

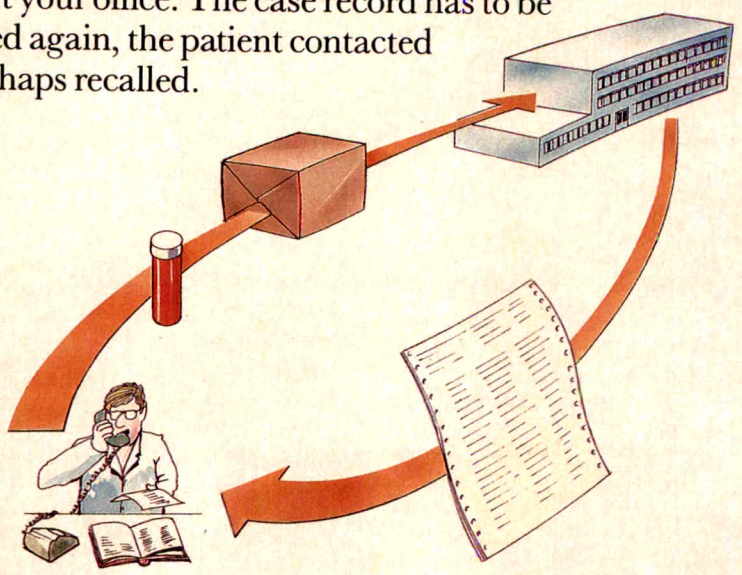
Hemoglobin determination, traditional procedures.

Traditional in-office Hb testing involves messy reagents, pipettes, test tubes, etc – not to mention instrument calibration and zero setting. A complex and time consuming procedure with high error potential, especially in the dispensing of blood and reagents.

Or take hematocrit where errors can arise from incorrect centrifuge speeds. And more to the point, why measure hematocrit when it is the Hb value that defines anemia?

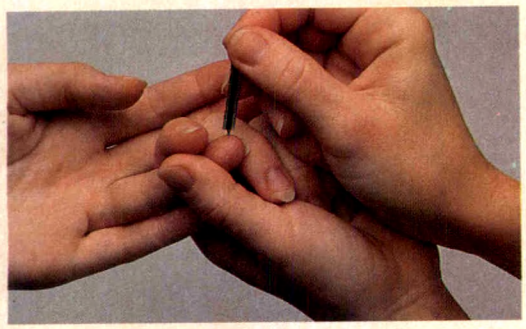


And what if you use an outside laboratory instead? When the test results finally arrive the patient has long since left your office. The case record has to be extracted again, the patient contacted and perhaps recalled.

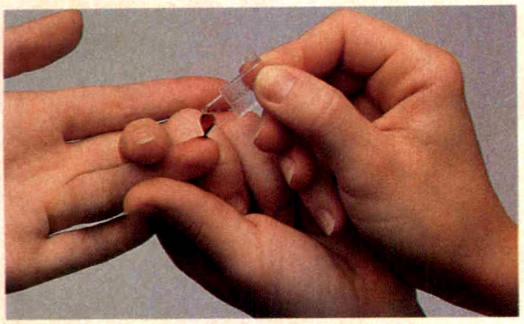


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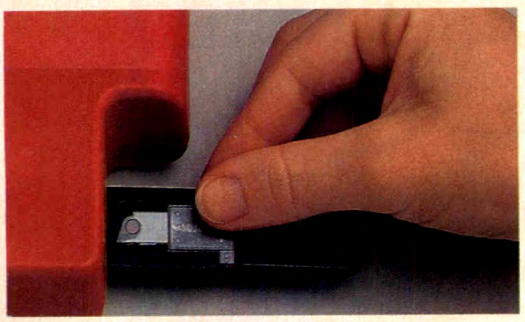
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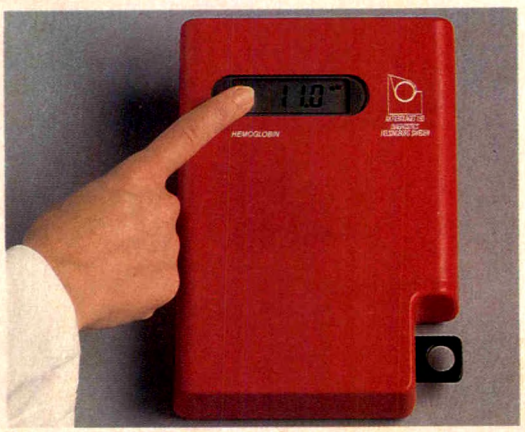
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Contents continued from page 13A

Longitudinal study of platelet indices during normal pregnancy 883
Steven G. Tygart, M.D., Dennis K. McRoyan, M.D., Joseph A. Spinnato, M.D., Cheryl J. McRoyan, M.T.(A.S.C.P.)S.H., and David Z. Kitay, M.D.
Mobile, Alabama

Aortic function during normal human pregnancy 887
Mark V. Hart, M.S., Mark J. Morton, M.D., Jeffrey D. Hosenpud, M.D., and James Metcalfe, M.D.
Portland, Oregon

Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings 892
Gamal H. el Tabbakh, M.D., Ibrahim Lotfy, M.D., Ibrahim Azab, M.D., Hany A. Rahman, M.D., Aaron L Southren, M.D., and Fatma A. Aleem, M.D., Ph.D.
Alexandria, Egypt, and Valhalla, New York

Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios 895
Adrien Bastide, M.D., Frank Manning, M.D., Christopher Harman, M.D., Ian Lange, M.D., and Ian Morrison, M.D.
Winnipeg, Manitoba, Canada

External cephalic version of the breech presentation under tocolysis 900
John C. Morrison, M.D., Ray E. Myatt, M.D., James N. Martin, Jr., M.D., G. Rodney Meeks, M.D., Rick W. Martin, M.D., Edsel T. Bucovaz, Ph.D., and Winfred L. Wiser, M.D.
Jackson, Mississippi

Prophylactic tocolysis of twins 904
James A. O'Leary, M.D.
Jacksonville, Florida

Epidemiology of hydatidiform mole during early gestation 906
Hani K. Atrash, M.D., Carol J. R. Hogue, Ph.D., and David A. Grimes, M.D.
Atlanta, Georgia

Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women 910
Ramiro A. Sánchez, M.D., Jorge E. Glenny, M.D., Enrique Marcó, M.D., Liliana S. Voto, M.D., Alicia M. Lapidus, M.D., Guillermo H. Iglesias, M.D., Luis V. Moledo, M.D., and Miguel Margulies, M.D.
Buenos Aires, Argentina

Sodium excretion in human pregnancy: A role for arginine vasopressin 914
Mark A. Brown, M.B., B.S., and Eileen D. M. Gallery, M.D.
Sydney, Australia

Contents

Profound hypotension complicating magnesium therapy 919
F. John Bourgeois, M.D., Siva Thiagarajah, M.D., Guy M. Harbert, Jr., M.D., and Cosmo DiFazio, M.D.
Charlottesville, Virginia

Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia 921
Susan K. Hendricks, M.D., Jonathan Keroes, M.D., and Michael Katz, M.D.
San Francisco, California

Ritodrine-induced neutropenia 924
Rebekah Wang-Cheng, M.D., and Bert J. Davidson, M.D., Ph.D.
Loma Linda, California

Meningoencephalitis caused by *Histoplasm capsulatum* complicating pregnancy 925
James A. McGregor, M.D., C.M., B. K. Kleinschmidt-DeMasters, M.D., and John Ogle, M.D.
Denver, Colorado

Sequential nonstress testing with use of each fetus as its own control 931
Lawrence D. Devoe, M.D., Ramon Castillo, M.D., Joan McKenzie, R.N., B.S.N., Nancy Searle, B.A., Beth Robinson, B.A., and Harry Davis, M.S.
Augusta, Georgia

Cesarean delivery of the second twin after vaginal delivery of the first twin 936
Pawan K. Rattan, M.D., Robert A. Knuppel, M.D., M.P.H., William F. O'Brien, M.D., and Jose C. Scerbo, M.D.
Tampa, Florida

Lactation suppression and puerperal fever 940
Oscar D. Almeida, Jr., M.D., and David Z. Kitay, M.D.
Mobile, Alabama

Atraumatic delivery in cases of malpresentation of the very low birth weight fetus at cesarean section: The splint technique 941
Maurice L. Druzin, M.D.
New York, New York

Cytosol estrogen receptor content of female parietal peritoneum 943
J. A. Hill, M.D., T. G. Muldoon, M.D., D. G. Gallup, M.D., W. A. Turner, M.D., R. A. Loy, M.D., and O. E. Talledo, M.D.
Augusta, Georgia

(Contents continued on page 19A)

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Contents continued from page 17A

SYMPOSIUM ON CEFOTETAN

Introduction 945
Richard Quintiliani, M.D.
Hartford and Farmington, Connecticut

Cefotetan in the treatment of obstetric and gynecologic infections 946
Alfred N. Poindexter III, M.D., Richard Sweet, M.D., and Marcella Ritter, M.D., Ph.D.
Houston, Texas, and San Francisco, California

Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section 951
Michael W. Varner, M.D., Carl P. Weiner, M.D., C. Rosemarie Petzold, B.S., and Rudolph P. Galask, M.D.
Iowa City, Iowa

Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients 955
James A. McGregor, M.D., C.M., Janice I. French, C.N.M., M.S., and Edgar Makowski, M.D.
Denver, Colorado

Cefotetan versus cefoxitin as prophylaxis in hysterectomy 960
James W. Orr, Jr., M.D., R. Edward Varner, M.D., Larry C. Kilgore, M.D., Robert C. Holloway, M.D., and Mary McDiarmid, R.N.
Birmingham, Alabama

CORRESPONDENCE

Another technique for resolution of shoulder dystocia Victoria Carter, *Seattle, Washington* 964

Prostaglandins and endometriosis C. S. Sgarlata, M.D., and F. Hertelendy, Ph.D. *Los Gatos, California, and St. Louis, Missouri* 964

Reply Michael J. James, Ph.D. *Bedford Park, South Australia* 964

Cigarette smoking and endometrial cancer John A. Baron, M.D. *Hanover, New Hampshire* 965

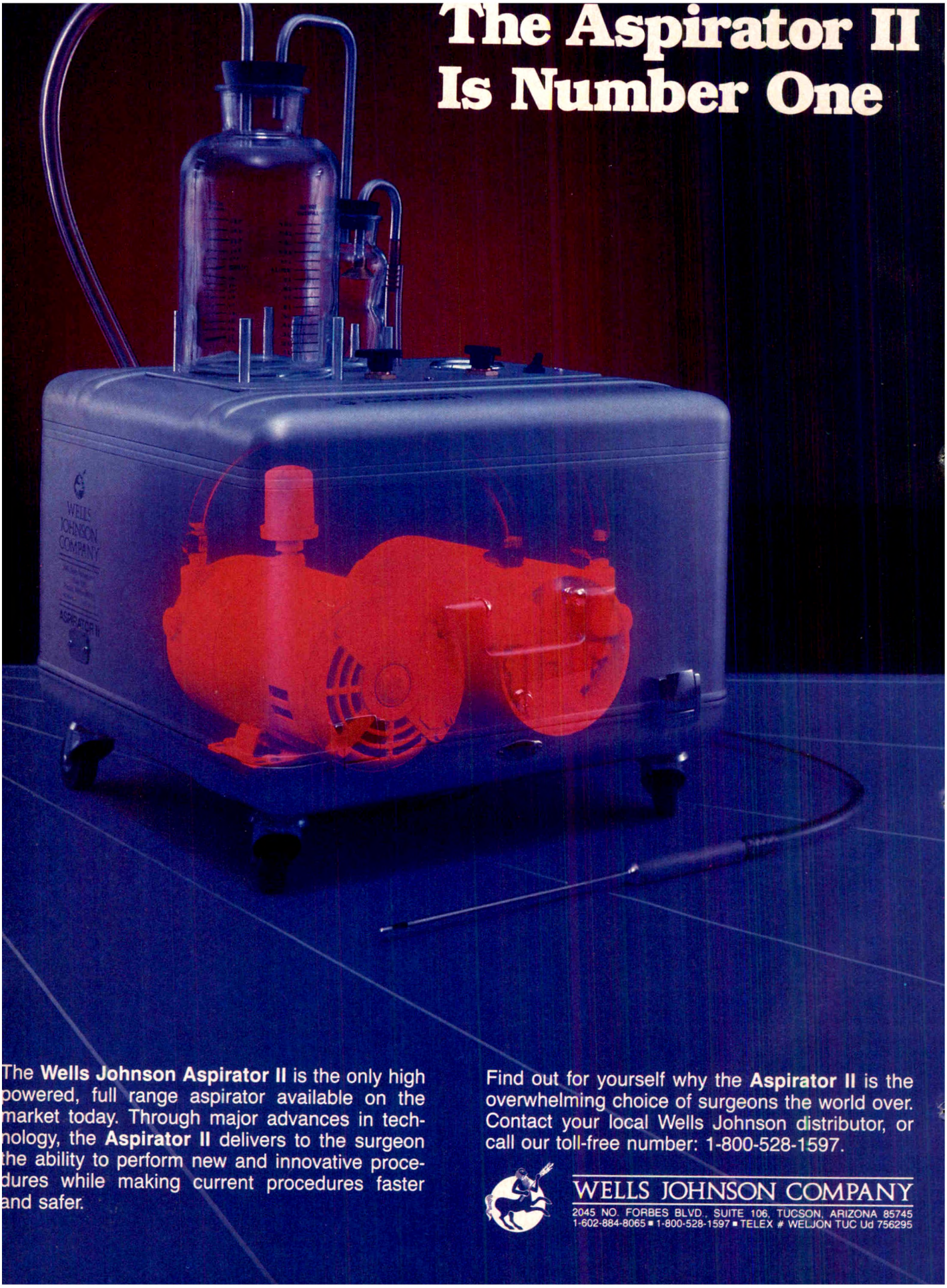
Testosterone levels and androgen-producing neoplasms David R. Meldrum, M.D. *Los Angeles, California* 965

Reply Chad I. Friedman, M.D. *Columbus, Ohio* 966

In defense of the vertical uterine incision Robert C. Goodlin, M.D. *Denver, Colorado* 966

Reply Radoslav Jovanovic, M.D. *New York, New York* 966

(Contents continued on page 21A)



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Contents continued from page 19A

Additional comment on combination conization Duane E. Townsend, M.D. *Sacramento, California* 967

Maternal and fetal surveillance for older pregnant women Arthur H. Schipul, Jr., M.D., Jerome N. Kopelman, M.D., and Patrick Duff, M.D. *Tacoma, Washington* 967

Reply Donna S. Kirz, M.D. *Chicago, Illinois* 967

Another view of controversy about Rh prophylaxis John D. Stephens, M.D. *San Jose, California* 968

A rapid method of culturing bloody amniotic fluid for chromosome analysis Jorge G. Rodriguez, B.Sc., Arvind Babu, Ph.D., and Ram S. Verma, Ph.D., D.C.C. *Brooklyn, New York* 969

Sterilization failures Richard J. Stock, M.D., M.P.H., Lieutenant Colonel, MC, USA *El Paso, Texas* 970

Reply Richard M. Soderstrom, M.D. *Seattle, Washington* 970

BOOKS

Books received 971

ITEMS

Items 972

Information for authors on pages 27A, 28A, and 29A

Index to advertisers on page 62A

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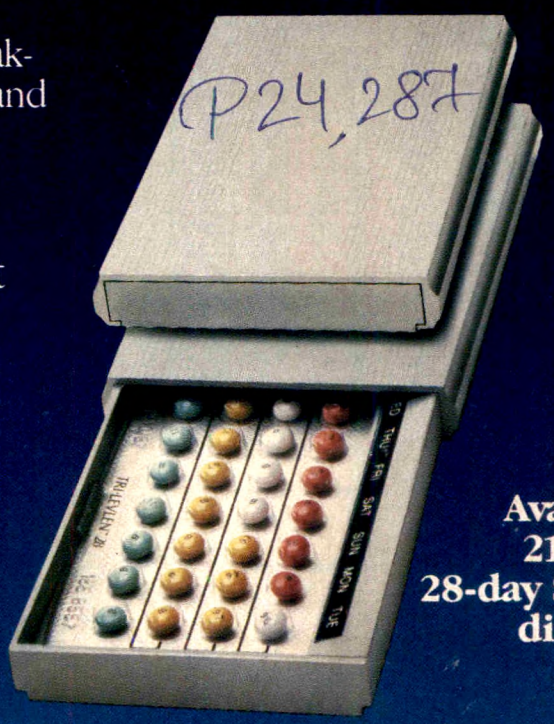
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Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted.: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. 2. Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985:153:495–500. 3. Briggs MH, in Brosens I (ed): *New Considerations in Oral Contraception*. Proceedings of an International Symposium. Leuven, Belgium (1981), 1982, pp 131-151.

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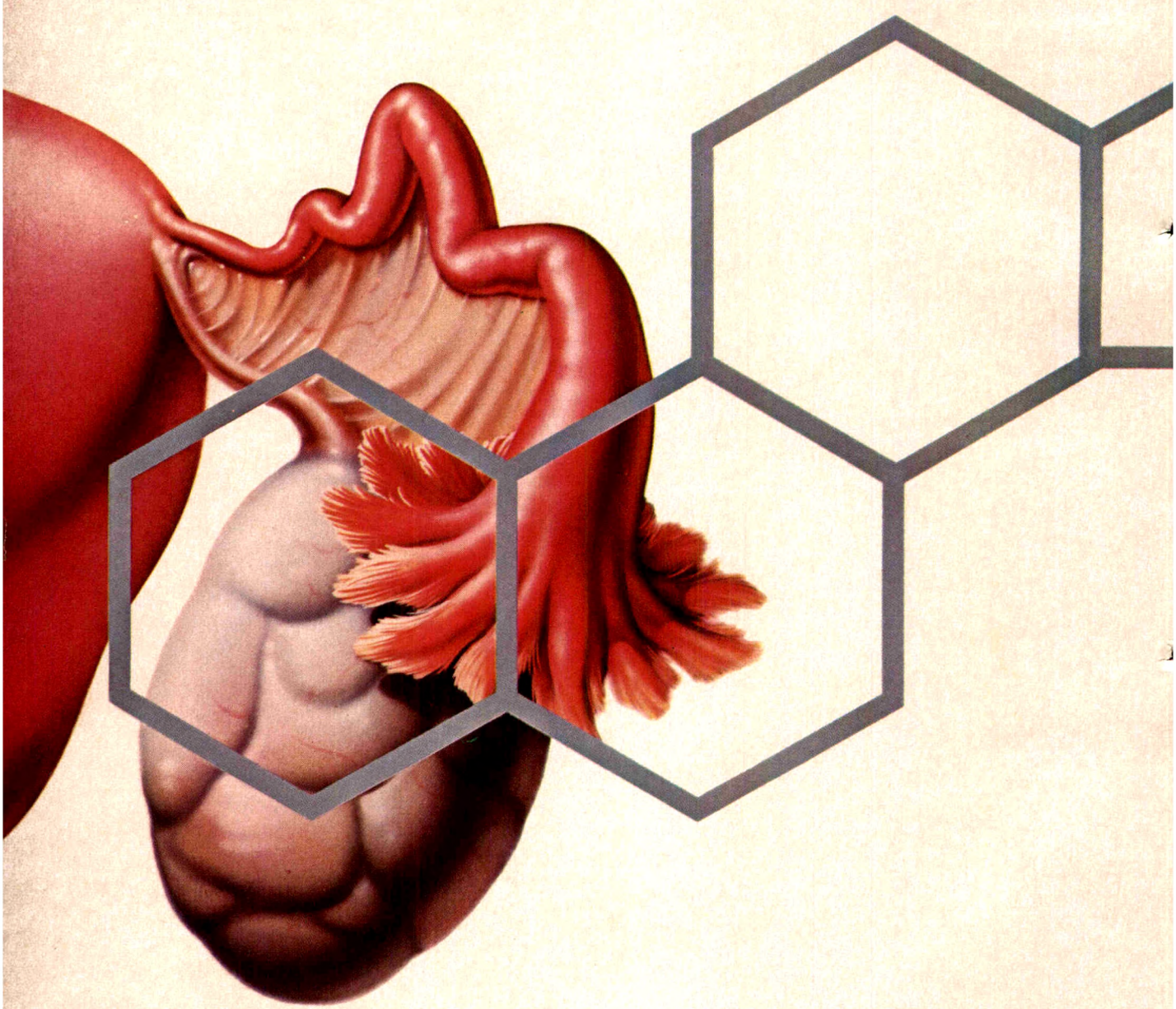
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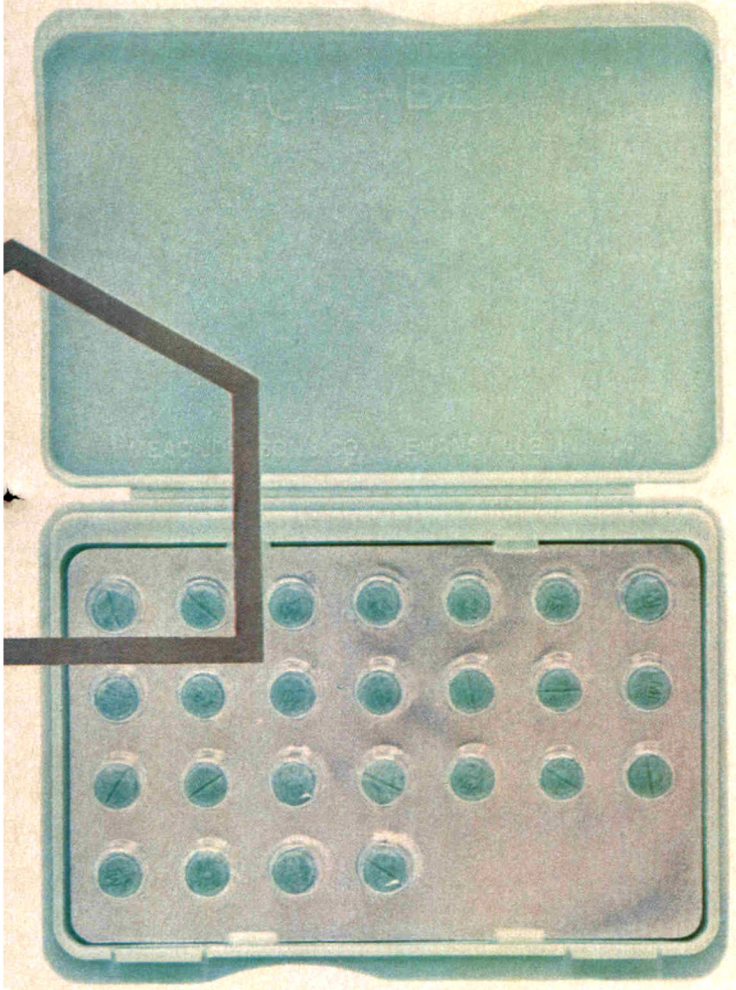
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2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Information for the Patient*—See text of Patient Package Insert. *Pregnancy Category X*—See Contraindications and Boxed Warning. *Nursing Mothers*—Administer to nursing mothers only when clearly necessary.

The 2-mg tablets contain FD&C Yellow No. 5 (tartrazine), which may cause allergic-type reactions (including bronchial asthma) in certain susceptible individuals, frequently those who also have aspirin hypersensitivity.

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Words, thoughts, and things

Presidential address

Joseph Seitchik, M.D.

San Antonio, Texas

I will take this opportunity to discuss two specific aspects of the relationship of our specialty to society. First, we will consider the words we use, presenting examples of how well they express or obscure our knowledge and our ignorance of some diseases and conditions we are expected to diagnose and treat. Second, we will identify changes in the practice of obstetrics in this century that represent responses by physicians to public criticism generated by women: most identified with or were sympathetic to the women's movement for social and economic equality. My observation is that physicians who practice obstetrics have always reacted to these pressures, that our responses were often inappropriate and accompanied by the appearance of new words and phrases, symbols that characteristically provided few clues to their precise meaning because they contained so little. In addition, I will present my views on the origins of the criticism and a few suggestions that I have the audacity to believe would reduce both the quantity and intensity of the complaints and aid in directing us toward appropriate responses.

"Words, Thoughts, and Things"; this is not original but is a chapter title borrowed from a book by Ogden and Richards[1] titled, *The Meaning of Meaning*. While reading literary criticism I found repeated references to it and my cowardly, but guilt-assuaging, response was always the same: "I'll save that one until I retire." I retired and, to my pleasure, I discovered that they were a pair of Cambridge University philosophers concerned with the problems created in communication between academics, because words used to express ideas are imprecise and suffer varying interpretations. When first published in 1923, their ideas must have been quite novel, for the book went through eight editions, the last in 1946.

Their proposition is simple: "Words as everyone now knows, 'mean' nothing by themselves . . . It is only when a thinker makes use of them that they stand for anything, or in one sense, have 'meaning.' " Take the word sequence: what shall we have for dinner mother. In your minds, place this phrase as a caption on a cartoon of a mother in a modern kitchen with a preadolescent child at her side. There is total coherence: in the words, the thoughts, and the things. Now if words have certain, precise, and explicit meaning, let us change the cartoon to a forest or jungle scene with a group of cannibals sitting around a very large pot over a fire. A head covered by a sunbonnet appears just above the edge of the pot, with the same caption, "What shall we have for dinner? Mother!" These are identical words in identical sequences but with totally different meanings.

According to Ogden and Richards,[1] the first step in separating the intellectual and emotive uses of language is to appreciate "the relationships of words, thoughts and things as they are found in cases of reflective speech uncomplicated by emotional, diplomatic, or other disturbances" (Fig. 1). Ogden and Richards use the word "referent" as we might use the word "thing," but because thing is usually used to denote material substances only, they adopted the word "referent," which stands for "thing, object, or entity." They teach that there is a strong relationship between things and

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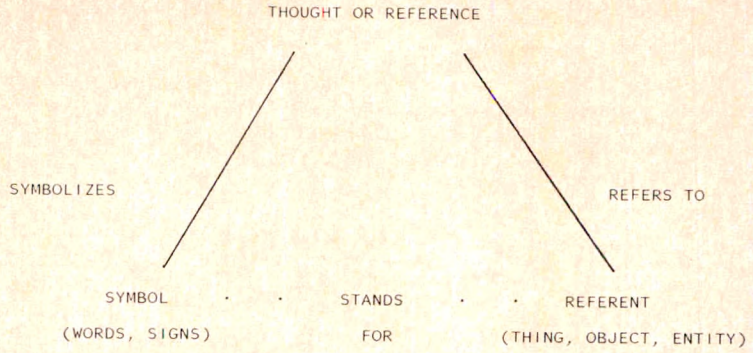


Fig. 1. The Ogden-Richards model of the relations of "words, thoughts, and things."

thoughts, referent and reference: causal relationships between words and thoughts, symbol and reference. But a word can only "stand for" an object; it cannot give meaning to it. They insist that if intellectual communication between humans is to be meaningful, we must differentiate between words, thoughts, and things. We must recognize that the emotive power of a single set of words and signs can generate dissimilar thoughts in different persons, which then produces inconstant referents. The result is that, except for a few onomatopoeic words, there is no cognitive association between words and things. An initial response to this whole proposition is, "it's obvious." Yet it is not. Think of the patient's response when the need for biopsy or colposcopy is announced. What causes her fear? Is it the phrase, "possibly malignant" (symbol), the idea of having cancer (reference), or the cancer itself (referent)? We recognize that it is not the disease she dreads, for the patient does not have the disease, the thing, the referent but suffers all of the terrible, anxious thoughts engendered by the symbol. This is one subject: how the interplay of words, thoughts, and things reflects the relationship of our specialty to society in general and to our clients, women, specifically.

Returning to *The Meaning of Meaning*, the book concludes with *Supplement II, The Importance of a Theory of Signs and a Critique of Language in the Study of Medicine,* by Crookshank. Crookshank was well known but unloved. After returning from service in France in World War I, he practiced in London, wrote a monograph on influenza (1922), which was well received by the profession, and started the Medical Society of Individual Psychology, which introduced Adlerian principles to the practice of psychiatry in England. He also wrote a dreadful book, titled *The Mongol in Our Midst,* presenting the preposterous hypothesis that the human race consists of several different species of different evolutionary origins. He graduated first in his class and was an accomplished debater who enjoyed bullying and embarrassing his opponents at public confrontations. His obituary in the *Lancet* reads, ". . . he displayed unusual erudition and a subtlety of argument which more than occasionally defeated its own purpose, requiring for its comprehension, a similar subtlety on the part of reader or audience. In argument consequently, Crookshank was too ready to blame the other side for failures to perceive what he had failed to explain . . .."[2] I hope that will not be the epilogue to my presentation today. Crookshank's complaints about physicians were twofold. First, he objected to physicians viewing diseases as entities in themselves as if they existed apart from the species *Homo sapiens.* Diseases must be viewed solely as pathologic processes with their manifestations in symptoms and signs. He argued that "diagnosis should mean the finding out of all that is wrong with a particular patient and why." He objected to the habit of the "formal and unctuous pronouncement of a name that is deemed appropriate and absolves from the necessity of further investigation." He wrote, "Medicine . . . has forfeited pretension to be deemed a Science because her Professors and Doctors decline to define fundamentals or to state first principles, and refuse to consider, in express terms, the relations between Things, Thoughts and Words involved in their communications to others."

This concept of the difference between thoughts and things was neither new nor unique to Cambridge dons. In Samuel Johnson's fable "Rasselas,"[3] the wise man, Imlac, attempts to educate Rasselas, an intelligent but young and inexperienced prince, to the difference between ideas and things. He says, "What space does the idea of a pyramid occupy more than the idea of a grain of corn?" William Carlos Williams,[4] general practitioner and poet, writes, "The poet does not . . . permit himself to go beyond the thought to be discovered in the context of that with which he is dealing: no ideas but in things." That was his battle cry, "no ideas but in things." The poet Wallace Stevens[5] wrote about ". . . that reality to which all of us are forever fleeing." This signifies that all intellectually serious and honest humans, not just philosophers and lexicographers and poets, must accept the responsibility to define reality with such clar-

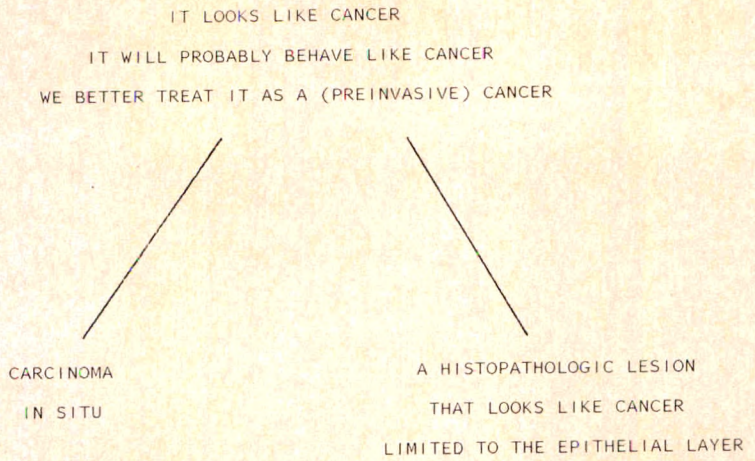


Fig. 2. Application of the Ogden-Richards model to an early view of preinvasive cervical epithelial lesions.

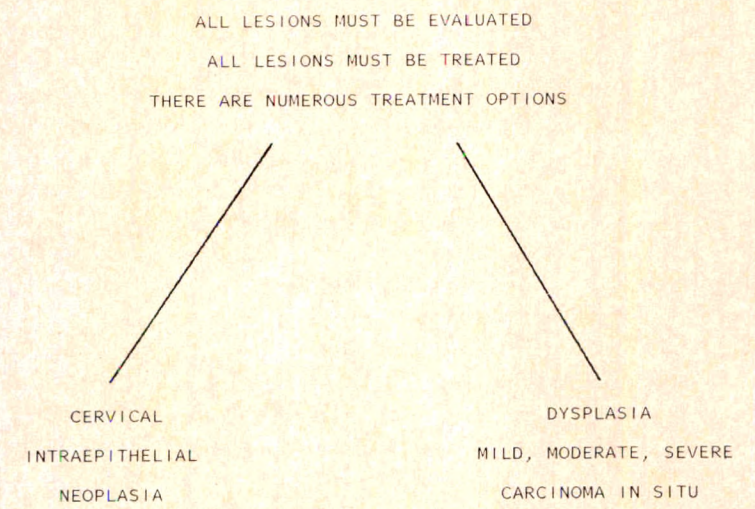


Fig. 3. Application of the Ogden-Richards model to the current view of preinvasive cervical epithelial lesions.

ity that our symbols evoke the same consciousness. It is my thesis that when we do, society is predisposed to accept our knowledge and leadership in health care. When we do not, we engender suspicion and hostility. I will give you a few examples, starting with a success.

With the recognition that epidermoid carcinoma of the cervix had a preinvasive phase called carcinoma in situ, physicians concerned with such problems provided words, thoughts, and things (Fig. 2). This formulation resulted in the performance of many hysterectomies and yet was accepted by society. We were praised for our efforts to identify and eradicate a major cause of death from malignancy in women. Yet that model was in part deficient and in part incorrect (Fig. 3).

What I wish to emphasize is that, as our knowledge of the lesion, the thing, expanded in both histopathologic and clinical dimensions, our words, the symbols, changed. Most important, the changes in language were consistent, not with the idea of the thing, but with the thing itself. As it was recognized that the lesion represented a histopathologic spectrum, including lesions that could regress spontaneously, cervical intraepithelial neoplasia replaced carcinoma, a change consistent with clinical realities. The words, thoughts, and things form a logical system. With all the complaints I hear and read about obstetrician/gynecologists, this is an area consistently avoided by our critics.

In contrast, the tripling of the cesarean rate in the 1970s resulted in the publication of many books critical of our practices and the formation of more than 30 lay organizations whose *raison d'être* is the protection of patients from our "unnecessary, aggressive practices."[6] If this increase in cesarean sections is beneficial to women and their neonates, we should be able to demonstrate the reality of the abnormalities that are the

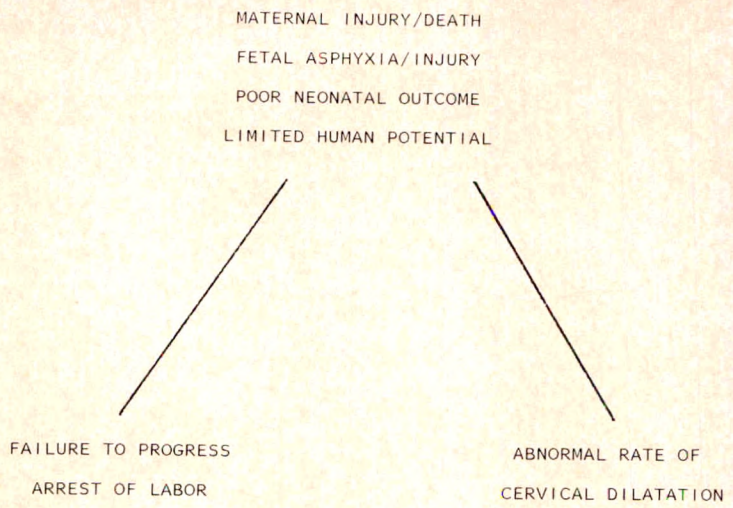


Fig. 4. Application of the Ogden-Richards model to the current view of functional dystocia.

causes for cesarean sections, the referent(s), the legitimacy of our thoughts, the reference(s), and the connotative specificity of the words we use, the symbols. Where shall we start?

Not in ideas but in things—what is the reality that resulted in the new ideas that produced more cesarean sections? Certainly no new obstetric disease has altered its occurrence from endemic to epidemic such as the venereal diseases—syphilis in the fifteenth century or acquired immune deficiency syndrome now. The specific anatomic dystocias such as contracted pelvis, brow presentation, and the like have decreased. The increase in cesarean sections is associated with the ascent of functional concepts of dystocia.[7] What happens when we apply the Ogden/Richards method of analysis to this problem? We start with the referent, abnormal rate of cervical dilatation and its symbols, failure to progress, and arrest of dilatation (Fig. 4).

If we are to be trusted, our words must reflect specific realities, not vague generalities. For those of us who experienced the great depression of the 1930s, "failure" was a frightening word with occasional suicidal sequence. After World War II, progress was our catchword. Economic progress would provide jobs for everyone. Progressive civil rights laws would assure equal opportunity for everyone. Thus "failure to progress" connoted something grave, something evil, when all it signified was some deviation from an average rate of cervical dilatation. The alternative phrase, arrest of labor, has a similar definition, but when used in a medical environment hints at associations with other arrests, cardiac for one. These are insinuating potentials for damage and death. The choice of these particular diagnostic symbols that reveal almost nothing, but have awesome, portentous connotations, should have aroused some suspicion that the phenomenon they purported to describe might be as ill-defined as the meaning of those phrases is delitescent.

Could it be that the words, the symbols, are poorly chosen but the events, the referents, all too real? How did we decide that episodes of no or slow cervical dilatation during labor are manifestations of pathologic processes rather than variants of normal processes? One standard, a minimum of 1 cm/hr and the need for action if the patient deviated from that rate for 2 hours, was developed as a rule of thumb for marginally trained midwives in Rhodesia to aid in identifying those patients who needed to be transported from their huts out in the bush to the hospital.[8] Other criteria for the definition of pathophysiology were developed as standard deviations from the means of the dilatation rates of large numbers of patients. What started out as a means of differentiating patients who were accomplishing their labor tasks rapidly from those less efficient and possibly in need of diagnostic reassessment became indications for special management techniques such as amniotomy, electronic monitoring, epidural anesthesia, and oxytocin therapy.

The severest criticism of the current cesarean rate is provided from three sources. Haverkamp et al.[9] challenge the need for monitoring and find that intermittent but frequent auscultation of the fetal heart rate suffices and that the elimination of electronic monitoring avoids escalation of the cesarean rate. O'Driscoll et al.[10] in Dublin insist that our high cesarean rate is unnecessary but substitute an aggressive system of care including a 40% incidence of oxytocin use in nulliparas. Finally, we have the newest feminist view of obstetrics.[11] To summarize their position, labor and delivery, although painful, can be a great source of pleasure, even with moments of ecstasy. Dealing with the pain is part of the business of being a woman who has chosen to

reproduce. Insecurity and anxiety make the pain worse; the support of an attentive father of the fetus and the assistance of a woman who has knowledge of the labor process are essential to success. These three systems of care are very different. One uses analgesics and anesthetics as required or requested; two do not. One insists that labor be rapid; two do not. One uses amniotomy and oxytocin frequently, one occasionally, and one not at all. All have similar cesarean rates on the order of 4% to 7%, depending on the mix of nulliparas and multiparas. They share only one common thread I can identify in their clinical practices. The common denominator is the continuous presence of bedside professional attendants.

Could the major cause of the increase in cesarean births from functional dystocia be so simple? Not that the monitor provides information that frightens the physician into inappropriate action as was once suspected but is now known to be untrue? Rather, these observations suggest that it is the absence of human support rather than the presence of the machine that is the villain. Whether my hypothesis concerning the mechanism whereby the new style of practice in labor and delivery increased the cesarean rate is correct is unimportant. What matters is that we cannot define a referent but only new thoughts that have increased the cesarean rate. What are these thoughts? Essentially they include that the increased cesarean rate will result in a reduction in perinatal deaths and that more cesarean births will produce more live and healthier neonates. What is the referent for these new practice ideas if they were not based on specific obstetric diseases? It is my thought that this change in practice was a response to public criticism, specifically to the complaint that the perinatal mortality rate in the United States was considerably higher than in several European countries. This habit of change in obstetric practice in response to public pressure has been and is typical of obstetric care in the United States in the twentieth century rather than extraordinary. Obviously, the events to which I refer are not those life-saving therapies designed for the treatment of specific disease entities—cesarean delivery for placenta previa, tracheal intubation for the prevention of meconium aspiration, or antibiotics for puerperal sepsis. No, I am referring to those styles of practice each of which, in its time, resulted from public pressure, each incorporated into obstetric practice, each eventually achieving public condemnation. I will review the evidence.

In the first decade of the twentieth century, German physicians introduced scopolamine and morphine as combination hypnotic/analgesic therapy for the avoidance of memory of the pain of labor. By 1914 to 1915, this method of pain relief was not widely used in the United States. Why not? Its decreased use was probably due in part to its complications, in part because most deliveries occurred at home and most patients could not afford the professional attention this treatment required, or perhaps most doctors were simply ignorant; we do not know. Mrs. C. Temple Emmet of New York, a granddaughter of the Astors, delivered in Freiburg, Germany, in 1909, experienced "twilight sleep," returned there for a repeat performance twice more, and, with her friends, initiated the National Twilight Sleep Association in New York City in 1915. This was a movement generated by upper-class club women and female physicians, for example, from Boston, Mrs. Jesse F. Attwater, editor of a magazine, *Femina,* and Dr. Eliza Taylor Ransom, physician and women's rights activist; from New York, Rheta Childe Dorr, author, suffragist, and trade unionist; and from Chicago, Dr. Bertha Van Hoosen, physician. None of these women is identified as general feminists, that is, leaders who recognized their gender as the basis for women's problems.[12] They were pragmatic feminists who identified specific areas of concern, such as the right to vote and exploitation of women and children in the workplace. It was not the radical feminists who concerned themselves with this issue, not the anarchists and socialists. It was upper-class women, in both wealth and education, who organized support for "twilight sleep." In the years 1914 to 1915, a series of articles appeared in the leading magazines of those days—*McClure's, Woman's Home Companion,* and *Good Housekeeping*—chastizing physicians for failing to provide twilight sleep to their suffering patients.[13a, 13b] These authors, Margaurite Tracy, Constance Leupp, Mary Boyd, and Hanna Ver Beck, were vigorous feminists seeking equal rights for women. They viewed twilight sleep as a means to equality. If women, like men, could avoid suffering the pain, agony, and terror of labor and delivery, one large difference in the experience of the sexes was deleted. The article in the *Ladies Home Journal* was accompanied by opinions the editors had requested from "eminent obstetricians." They were J. Whitridge Williams of Johns Hopkins, Charles M. Green of Harvard, Barton Cooke Hirst of the University of Pennsylvania, and Joseph B. DeLee of Northwestern University. All four condemned the use of twilight sleep. Nonetheless, under these pressures, physicians adopted twilight sleep. The effects were obvious: more hospitalization for delivery, but only for those who could afford it or the poor, an increasing role for physicians in the birth process, and a diminished role for the patient in her care. Physicians responded to this demand even though the deleterious effects of twilight sleep in slowing labor and inhibiting respiration in the newborn infant were obvious to those "eminent obstetricians" who had examined its effects objectively. We should not view this episode as a desire for women to seek less control of the childbirth expe-

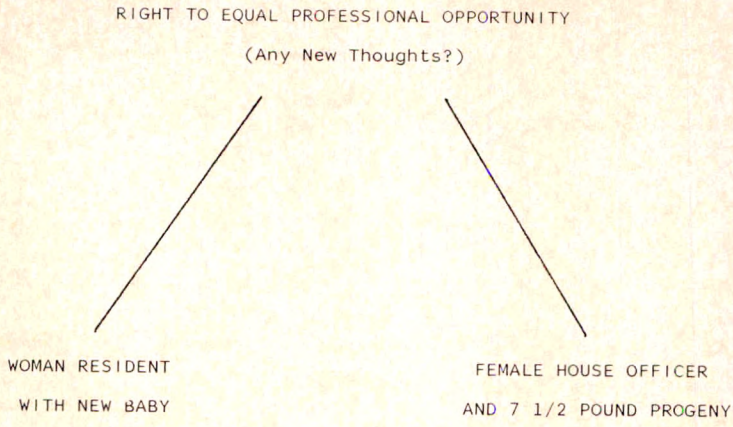


Fig. 5. A derisive and incomplete application of the Ogden-Richards model to maternity in residents.

rience. These women wanted more control. The fact that the "control" resulted in their amnesia is of no significance. They wanted to labor and deliver by a method of their choice.

Shortly after this outcry and response, another major innovation occurred in obstetric practice. By the end of the second decade, Dr. Joseph B. DeLee had introduced his system of care for all laboring women,[14] including normal obstetric patients without complications. In essence, it was an exercise in applying the methods of surgery to obstetrics. All patients had the genital areas shaved, all patients were draped in sterile vestments, all patients were scrubbed with antiseptics, all patients underwent a routine episiotomy, and all babies were delivered with routine low forceps. The goal of this system was not the treatment of any specific disease but an attempt to reduce maternal injury and death by the application of the principles of surgery to obstetric delivery in a hospital environment. Who was concerned with the maternal mortality rate? Not only were Dr. DeLee and his obstetric colleagues concerned but also Dr. Grace L. Meigs.[15] Dr. Meigs received her A.B. degree from Bryn Mawr College in 1903 and her M.D. degree from Rush Medical College of the University of Chicago in 1908. After interning at Cook County Hospital, she practiced in Chicago until 1914, when she moved to Washington, D.C. to become the Director of the Division of Hygiene of the Children's Bureau of the Department of Labor. In 1917, she produced the first official survey of maternal mortality rates in the United States, discovering that for women aged 15 to 44 years, maternity was the leading cause of death after tuberculosis. In a comparison with 15 other countries, including such different places as New Zealand and Spain, none had as many maternal deaths per 1000 live births as we. It is important to note that the language used by Dr. Meigs to identify the causes of this high maternal mortality rate was almost identical to that of J. Whitridge Williams.[16] To paraphrase them both: The problems were twofold: (1) Skilled obstetric care was not valued, so obstetric fees were very small; (2) physicians were also reluctant to assume the responsibilities of obstetric care because they were so often required to accept difficult cases of labor outside hospitals and without any nursing assistance. I must digress for a moment to contrast the situation as they described and recorded it at that time with the history of obstetrics in the United States, which is so popular with our academic critics from the humanities. In his book *Power and the Profession of Obstetrics*, William Ray Arney,[17] after assuring us of his objectivity and his recognition that there are two histories of obstetrics, one by us and another by our critics, he writes, "It (his book) uses the historical archive of the profession to examine the problems of, first, how the profession seized childbirth and staked it out as the exclusive domain of a new profession; . . .." First, note the word choices, "seized childbirth" and "staked it out." Surely this is language more suited to war or revolution than a description of evolutionary events occurring over a period of 2 centuries. Further, if the description relates specifically to the turn of the century in the United States, the writings of Dr. Meigs from the Labor Department and Dr. Williams at that time clearly indicate avoidance of obstetric cases by the vast majority of physicians—hardly "seizing" and "staking out."

The response to Dr. Meig's work seems limited to the specialty of obstetrics. I could find no newspaper or magazine article concerned with any aspect of maternal deaths in the year 1917. No editorial appeared in the *Journal of the American Medical Association*. The *New York Times* had many reports on women and war work, the suffragettes, and even on the women's peace movement but none on maternal mortality in the year 1917. The matter was not ignored by obstetricians. Dr. Meigs[18] addressed the New York Academy of Medicine, Section on Obstetrics and Gynecology, by invitation, on May 22, 1917. The transactions of the meeting were pub-

lished in the *American Journal of Obstetrics and Diseases of Women and Children,* and an editorial followed.[19] All the discussants agreed with the importance of her work and the need for action. The editorial is interesting in several respects. In analyzing the relative failure to reduce the number of maternal deaths during a period of relative success in reducing death rates from tuberculosis, typhoid, and diphtheria, the author writes ". . . the problem is vastly different . . . when one takes into consideration the numerous social and economic, in addition to the purely medical phases involved." The editorial paraphrases the various solutions suggested by "competent medical observers." They are: (1) improved teaching of obstetrics to physicians; (2) proper instruction and supervision of midwives; (3) adequate prenatal care and hospital facilities, particularly for women of moderate means; and (4) state grants of money to mothers of families who may require such aid. This does not seem to me to be a battle cry for the medical profession to "colonize childbirth." That quote, "colonize childbirth," is from the work of one of our English critics, Ann Oakley,[20, 21] who has authored *Women Confined: Toward a Sociology of Childbirth* and *The Captured Womb: A History of the Medical Care of Pregnant Women.*

Returning to Dr. DeLee's attempts to improve obstetric outcome by the application of surgical techniques to delivery, I cannot be certain that this was a response to the reality of Dr. Meig's data—the fact that maternal mortality rates had not declined from 1900 to 1913. Whatever his motivations, the truth is that Dr. DeLee's good intentions accomplished little in reducing maternal mortality rates. Abrupt decreases in maternal death rates have occurred only in association with new developments in medical science. The first major decline occurred in the 1930s in association with the general use of blood banking and the sulfonamides and the second following the availability of penicillin and blood products immediately after World War II. The maternal death rate remained almost constant from the time of Meig's report in 1917 to 1932 at 65 to 70 per 10,000 births, or approximately one maternal death per 150 deliveries.

If one were to look at the four decades from 1910 to 1950 and ask, "What can we learn from that period of obstetric history?" I would reply that it demonstrated that the best interests of maternity patients were served when our best current knowledge of medical science was applied in obstetric care. Further, it teaches that, at any singular time, our knowledge of medical science is finite. Significant new developments necessary to solve our patients' problems will come in their time but are not at our mere beck and call. When faced with a public challenge, we have responded by extending the application of the medical tools available to us without applying the scientific method to the analysis of their potential costs and benefits. This is our "as if" response. When criticized, we have sought responses to these challenges in terms of inadequately tested hypotheses or techniques. "As if" depriving mothers of any memory of childbirth, both the pleasures and the pain, will make women social and economic equals of men. "As if" applying the techniques appropriate to the surgical amphitheater is advantageous for laboring women. What this history teaches us is that every time these critical Eves have offered us a bite of the apple of complaint, we have gorged ourselves on all the fruits of technology. Some of our modern critics insist that we and our predecessors initiated the use of these therapies because progressive reliance on the physician to control the circumstances of birth assured physicians financial benefit. I deny that but would also insist that our responses have not been invariably consistent with the best health interests of our obstetric patients. It is a wonderment to me why we have not invested in understanding these public pressures and identifying and appreciating their origins instead of reflex responses that use medical technologies of uncertain value or safety. This pattern of reflex technologic response continues. We are now completing our most recent cycle. Having responded to the criticism of the 1960s, we increased the cesarean birth rate "as if" that would cause significant improvement in the quality and numbers of live-born infants—another response to public pressure in the enhanced use of medical technology without a clear, objective demonstration of its value. These events were followed by increasing criticism of this behavior from the public and government. Now clinical evidence indicates that recent cesarean rates were excessive, and I suspect that the numbers of cesarean births are declining. The cycle nears completion.

Could we eliminate this criticism if all our practices were scaled thoroughly by application of the scientific method before use? I think not, because the problem is rooted in biology. If, by some miracle, we should change our history and provide women with every equal right in every area of human endeavor, the one remaining, intractable difference between men and women would be pregnancy and the labor/delivery experience. If nature provides us with this difference between males and females in the production of the neonate, what are the legitimate derivatives in nurture?

Can Ogden and Richards help us? Let us start with a simple example. The referent is a female house officer. The symbol is a woman physician, and I believe that, on all teaching services today, the thoughts are of equality: equal opportunity for education and training and practice or academic career. Now let us complicate the diagram (Fig. 5).

Are there any new thoughts? I believe there are. Until

1981 neither the Residency Review Committee nor the American Board of Obstetrics and Gynecology found it necessary to provide a national standard for sick leave and vacations. I presume these organizations thought such matters were best managed at a local level with the director of the residency program making these judgments. But in 1981 a new rule appeared in the American Board of Obstetrics and Gynecology bulletin, limiting total absence for nonprofessional activities to 6 weeks per year. Greater absence requires compensatory makeup time. I have struggled to identify the recent epidemic of disease among obstetric and gynecologic residents that has stimulated this new regulation but can find none. Perhaps it is unfair to relate this action to the fact that women physicians are or soon will be a majority among our residents. But the history of women is so rife with these examples of loss of equality on childbirth, it is difficult for me to avoid suspecting this cause-and-effect relationship.

While the seed of the problem is in nature, how it germinates and what the seed produces is in nurture. How will the "equality" of our reference be maintained if the problem of childrearing is the mother's major responsibility? There is no way she can avoid constriction of her educational opportunities and eventually diminished career and income. Would it not have been a great symbolic leap forward, promoting mental health and equality, if the Residency Review Committee and American Board of Obstetrics and Gynecology issued a statement that read like this: "Because only women have the unique capacity to produce new humans, an ability we value as a society, every effort should be made to assist those female residents who decide to help renew the human race by having children during their residencies. We recommend that every hospital establish 24-hour nurseries for the residents' children so that they may return to work as soon as possible and can continue to nurse. We believe nursing is important to the health of these babies."

This paradigm is a small example of a very important issue: How do we compensate for the career limits placed on women because of the arrival of children or how do we avoid these limits? We have an enormous range of opinion.[22] Some think this is a responsibility of the nuclear family. Others think that society must invest in maintaining equal opportunity for women post partum, particularly in these days of more nuclear family fission than fusion. Others believe that women will be able to have children without loss of their personhood only when men change their orientation to acceptance of equal responsibility for child rearing. What is the relationship of all this to our critics?

While it may seem bizarre, examination of the bulk of these publications reveals that the majority of our critics are women who have resolved this particular problem. The "twilight sleep" movement was led by club women so wealthy that some not only went to Freiburg to have their babies but stayed on for months because they did not want their babies to change "wet nurses." They could buy any arrangements they wished. A major source of criticism continues to come from women whose education and incomes far exceed those of average men and women. Most are professionals: writers, nurses, and academics. They appear to be successful in their careers, incomes, heterosexual relationships, and child rearing. They have the money, the talent, and the knowledge of resources necessary to adapt to motherhood without loss or constriction of their careers or incomes. They also have the money necessary to create their own environments in labor-delivery suites that provide traditional obstetric care. *A Good Birth, a Safe Birth* is written by two such women.[6] Among their recommendations are:

> Summary: How to have a normal birth and avoid an unnecessary cesarean.
>
> Labor
>
> 6. Hire a monotrice to monitor your labor and your baby's heart rate, especially if that is the only way you can avoid a fetal monitor.
> 9. Have at least one woman you know and like with you—your doula—in addition to your husband.

This provides the patient, who senses control over every aspect of her life, control over the labor and delivery process as well. Lest you think I am making a major issue of an isolated publication, similar attitudes are found in *Immaculate Deception. A New Look at Women and Childbirth in America* by Suzanne Arms.[23] Unfortunately, there is a fair amount of deception in the book. Another is *Silent Knife. Cesarean Prevention and Vaginal Birth After Cesarean* by Nancy Wainer Cohen and Lois J. Estner.[24] Both of these women have experienced vaginal delivery after an initial cesarean birth. Another example is *How to Avoid a Cesarean Section* by Christopher Norwood.[25] I avoid referring to this as "yuppie obstetrics," because identical goals are also found in a totally different group of women. Ina May Gaskin[26] wrote *Spiritual Midwifery*. She was the first lay midwife at "The Farm" in Summertown, Tennessee, a vegetarian agricultural commune whose spiritual orientation is in the Zen tradition. Its original members were largely drop-outs from the drug and hippie life who accepted Zen and emigrated to rural Tennessee to create a successful self-supporting community. They describe themselves as follows: "We are not just a community. We are a church. We hold our land in common, and share fortunes. No money is exchanged for goods or services among the people of our community." Their

obstetric principles are identical, in respect to patient and lay midwife responsibilities, to those of our upper-class critics. On "The Farm," becoming a mother produces no undesirable changes in life-style or social or economic status.

Women who do not compromise their goals because they become mothers represent the major source of our lay critics. We might have speculated that the opposite would be true. The explanation of this apparent contradiction seems clear. Women who have control over their life-styles, particularly control of the impact of children, also wish to have control over childbirth as well. Women who do not have the resources or support to avoid the negative impact of child rearing have the much more serious problem of loss of control of their lives. The importance of the events of 12 hours of labor pales for them when contrasted with the many years of loss of their own growth potential. Recognize that our critics do not wish any change from their mode of living, whether in the farm commune or "yuppieland." These are not radicals in the sense that they wish to change the structure of society—only to dominate their own environments. The radical critics of our specialty have little to say about obstetric practice. Mary Daly[27] was an associate professor of theology at Boston College in 1978 when she published *Gyn/Ecology, the Metaethics of Radical Feminism.* Two successive chapter headings are labeled: Chapter Seven. American Gynecology: Gynocide by the Holy Ghosts of Medicine and Therapy and Conclusion and Afterword to Chapter Seven. Nazi Medicine and American Gynecology: A Torture Cross-Cultural Comparison. Because some of you will not rush to read the book, I will tell you the ending of this section. We came out in a tie with Dr. Mengele. More important, she is sympathetic to feminists who "wish to explore the possibility of parthenogenesis," potentially realistic these days with the availability of unfertilized ova in the laboratory. She would then achieve her "final solution," which in another frame of reference she calls a "mister-ectomy." In spite of this intense hatred of men in general and male gynecologists specifically, I could find only four sentences concerning obstetrics in 424 pages of text. Three complained about the use of scopolamine in the present tense, and the fourth was this inexplicable comment on natural childbirth: "Natural childbirth . . . is nothing more than a romanticized means of helping women to better adjust to the abnormal and intensely painful delivery process mandated by men." No alternative suggestions for practice are offered.

If my hypothesis is correct, if the wish for self-reliance in childbirth is in the main important to women whose life goals will not be altered significantly by a neonate, what about the rest? Most women do not live in communes or have the incomes, education, support systems, and sophistication to avoid the limiting effects children place on their lives. For example, a little more than one fifth of our patients are single, widowed, or divorced at the time they are delivered. Few have a "significant other." I believe that is the current term for father but not husband. In Korte and Scaer's book,[6] the word "husband" is used only with positive connotations such as the importance of the husband's presence during labor. The term "father" is used when the male fails to provide proper support. Of course he fails because the hospital or physician would not allow him to participate, never because he was long gone those many months. No "yuppie" father/husband would do such a thing. I think it is pointless to detail the specific differences between the "yuppie" or "The Farm" women and the bulk of our patients. All of the factors that will cause the vast majority of women to alter their life-styles after childbirth also interfere with their ability to succeed in achieving nonmedicated births consistently. This is no reason to deny them education or preparation for childbirth. It never occurs to Mss. Korte, Scaer, Gaskin, Arms, Cohen, Estner, or Norwood that there is a whole imperfect world out there of women to whom the fates have not been as kind: women who, for a variety of reasons, are not as prepared to meet the responsibilities of pregnancy and delivery. I find no evil in using our professional knowledge and skills to help them through labors they find painful and threatening. This is an issue our highly educated critics, blessed with the life-styles they want, ignore.

In summary, we must be certain that the words we use connote the same meaning to all, that our symbols specify, not obscure. Past and present critics of obstetric practice in the United States in this century are women whose life-styles are not injured by childbearing or rearing. These women who control the particulars of their lives wish to control the circumstances of childbirth as well. When their desires are consistent with our knowledge of medical science we should admire their independence and be supportive; when inconsistent, we should have the courage to reject the criticism without renouncing the critic. If my hypothesis that there is a positive relationship between female equality as so defined and the acceptance of personal responsibility for childbirth is correct, then the bulk of women in our society will expect to receive our help even when no specific obstetric pathology exists. We must not continue to respond to criticism by extending the use of our diagnostic and therapeutic technology beyond its proved usefulness as tested by the scientific method. Finally, we know we do not have either the power or the desire to "seize, stake out, or colonize" childbirth or the ability to remove all the limits society places on

women, but we can protect our own female professionals from the potential career constraints of childbirth and childrearing.

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Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis

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Further purification of a porcine follicular fluid fraction, referred to as follicle regulatory protein, that inhibits granulosa cell aromatase was performed and the results of in vitro bioassays with these highly purified reagents are reported. The 0% to 35% saturated ammonium sulfate extract of porcine follicular fluid was percolated through an orange A dye matrex gel column and the bound fraction was eluted. Further purification of 0% to 35% orange A–bound fraction of porcine follicular fluid was performed by anion exchange chromatography with the use of the Mono Q column. Mono Q eluents containing follicle regulatory protein activity were injected onto a Mono P hydrogen ion–exchange column. Samples obtained from Mono P chromatography were injected onto preparative and analytical scale gel exclusion columns. Eluent fractions in the apparent molecular weight of 16,000 daltons were tested for aromatase inhibition. Throughout each step, parallelism of an aromatase inhibitor was apparent in both a cell-free microsomal assay and a granulosa cell assay. Follicle regulatory protein, purified about 6666-fold from the orange A–bound fraction of porcine follicular fluid, had a 50% inhibitory concentration of 25 ng/ml for granulosa cell aromatase activity. (Am J Obstet Gynecol 1986;154:709-16.)

Key words: Follicle regulatory protein, granulosa cell, aromatase

The dynamics of follicle recruitment and growth are not strictly functions of time in that they are controlled, in part, by intraovarian events. We sought to examine how the preovulatory follicle maintains its dominance over other follicles in either ovary as well as how this dominance may participate in the preservation of the ovulatory quota. We found evidence that the graafian follicle produces and secretes a hormone-like protein, referred to as follicle regulatory protein, which affects the responsivity of other follicles to gonadotropin stimulation.

Follicle regulatory protein is secreted by human[1, 2] and porcine[3] granulosa cells and does not inhibit secretion of follicle-stimulating hormone by pituitary cells in vitro.[4, 5] Therefore, follicle regulatory protein has biologic properties different from those of inhibin. Follicle regulatory protein has been shown to: (1) inhibit the increase in ovarian weight and serum estradiol levels in response to human menopausal gonadotropin in hypophysectomized, immature, diethylstilbestrol-treated rats[6, 7]; (2) delay follicular maturation into regularly cycling monkeys as evidenced by decreased serum estradiol levels, prolongation of the follicular phase, and anovulation or luteal phase dysfunction in the presence of normal serum gonadotropin levels[8]; (3) inhibit follicle-stimulating hormone induction of luteinizing hormone–human chorionic gonadotropin receptors in porcine granulosa cells[9]; (4) inhibit follicle-stimulating hormone–responsive adenylate cyclase activity in porcine granulosa cells[10]; and (5) inhibit in vitro microsomal aromatase[11, 12] and 3β-hydroxysteroid dehydrogenase activities,[12] thereby altering production and secretion of progesterone from granulosa cells.[13-16] This follicular regulatory protein is not toxic to granulosa cells in vitro and its effects are reversible.[11, 13]

The studies listed above were performed with a follicle regulatory protein reagent prepared by ammonium sulfate precipitation, followed by dialysis and further fractionation with the use of Matrex gel orange A chromatography. Here, the further purification of follicle regulatory protein and the results of in vitro bioassays performed with these more highly purified reagents are reported; these address the physiologic role of follicle regulatory protein and the process of dominant follicle selection in women.

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Material

A fast protein liquid chromatography system consisting of a GP-250 gradient programmer, two P-500 high-precision pumps, a UV-1 single path ultraviolet monitor, a pH monitor, a flow-through pH electrode, a FRAC-100 fraction collector, an REC-482 two-channeled recorder, a V-7 valve injector, a 10 ml Superloop, and a 50 ml Superloop as well as an HR 5/5 Mono bead anion exchange Mono Q column (0.5 by 5 cm), an HR 5/20 Mono bead hydrogen ion exchange Mono P column (0.75 by 30 cm), and molecular weight standards was obtained from Pharmacia, Inc., Piscataway, New Jersey. A Gilson (Middleton, Wisconsin) FC-80K fractionator and an ISCO (Lincoln, Nebraska) Model UA-5 detector were also used.

High-performance liquid chromatography included an M-730 data module, a Model 720 system controller, Model 6000 A and M45 solvent delivery systems, a Model 450 ultraviolet variable wavelength detector, and an automated 710 B sample processor and Diaflo membrane (PM10, molecular weight 10,000 cutoff) purchased from Amicon (Lexington, Massachusetts). The Bio-Sil TSK-250 analytical gel permeation column (0.75 by 60 cm, TSK analytical column) was provided by Bio-Rad (Richmond, California). The Spherogel TSK-G3000SWG preparative column (2.1 by 60 cm), Spherogel TSK-Guard column (2.1 by 7.5 cm), and pH monitoring equipment were obtained from Beckman Instruments, Fullerton, California. All chemicals and reagents were purchased from Sigma Chemical Co., St. Louis, Missouri, and radioisotopes were obtained from New England Nuclear, Boston, Massachusetts. Bufferlyte, pH 4 to 8, was provided by the Pierce Chemical Co., Rockford, Illinois. Dialysis tubing (6000 to 8000 molecular weight exclusion) was obtained from Spectrum Medical Industries, Los Angeles, California. Pooled porcine follicular fluid was provided by Dr. Gabriel Bailey of the Contraceptive Development Branch, National Institute of Child Health and Human Development, and fresh porcine follicular fluid was aspirated from porcine ovaries obtained from a local slaughterhouse. All procedures were done at room temperature unless otherwise stated.

Methods

Ammonium sulfate precipitation of follicular fluid. From a stock of frozen porcine follicular fluid, 1 L was slowly thawed and centrifuged at 4° C in a Sorvall RC-2B at 5000 rpm for 30 minutes to remove particulates. To the supernatant, 320 gm of crystalline ammonium sulfate was slowly added under gentle stirring in an ice bath. This solution was slowly stirred for an additional 2 hours and centrifuged at 5000 rpm for 30 minutes at 4° C. The precipitate was resuspended in 1000 ml of chilled 35% saturated ammonium sulfate solution, stirred for 2 hours in an ice bath and allowed to stand at 4° C overnight. The precipitate (0% to 35% saturated ammonium sulfate) was obtained by centrifugation. The precipitate was dissolved in 0.05 mol/L Tris hydrochloride, pH 7.5, and 0.1 mol/L sodium chloride (buffer A), dialyzed against three changes of distilled water, and centrifuged. The supernatant fraction was stored at −20° C.

Dye matrex orange A chromatography. A 100 ml charge containing approximately 10 gm of the 0% to 35% saturated ammonium sulfate extract of porcine follicular fluid was slowly percolated through an orange A matrex gel column (4 by 25 cm, 300 gm of dye matrex gel orange A) equilibrated with 0.02 mol/L Tris hydrochloride, pH 7.5 (buffer B) at 4° C. After loading, the column flow was halted for 1 hour to allow binding to occur. Unbound material was eluted with approximately 600 ml of buffer B at a flow rate of 1 ml/min and the bound fraction was eluted with 400 ml of 0.5 mol/L potassium chloride in buffer B. Fractions of 5 ml were collected. Fractions containing activity were pooled, dialyzed against distilled water, and lyophilized. This lyophilized fraction is referred to as 0% to 35% orange A–bound material.

Separation of follicle regulatory protein from 0% to 35% orange A–bound material by anion-exchange chromatography. The 0% to 35% orange A–bound material was further purified by anion-exchange chromatography with the Mono Q column by a fast protein liquid chromatography system. Gradient elution was controlled with a GP-250 gradient programmer. All buffers were filtered through 0.22 μm cellulose acetate filters (HAWP 04700) and then degassed prior to fast protein liquid chromatography. For each Mono Q run, 20 mg of 0% to 35% orange A–bound material dissolved in 1 ml of buffer B was applied as a charge to a Mono Q column previously equilibrated with buffer B. After loading, the column was washed with 5 ml of buffer B to elute unbound material at a flow rate of 1 ml/min and the bound material was eluted with the use of a linear gradient of sodium chloride in buffer B (0.0 to 0.4) mol/L during a period of 20 minutes. The column was stripped by means of a 2-minute wash with 1 mol/L sodium chloride in buffer B and then reequilibrated with starting buffer. Eluent fractions that contained biologic activity were pooled and dialyzed against distilled water to remove salt and lyophilized. Mono Q eluents containing follicle regulatory protein activity are referred to as Mono Q follicle regulatory protein.

Separation of follicle regulatory protein from Mono Q follicle regulatory protein by hydrogen ion–exchange chromatography. Twenty milligrams of Mono Q follicle regulatory protein was dissolved in 0.025 mol/L *bis* Tris hydrochloride, pH 7.1 (buffer C) and

injected with the previously described fast protein liquid chromatography system onto a Mono P hydrogen exchange column previously equilibrated with buffer C at a flow rate of 1 ml/min. After the column was washed with buffer C for 10 minutes, the sample was eluted isocratically with 50 ml of Pierce Bufferlyte 4-8 (diluted 1:20 in distilled water and adjusted to pH 4.0). A 1 ml aliquot of buffer C containing 2 mol/L sodium chloride was injected onto the column and elution with buffer C was continued for an additional 10 minutes. The pH values of all fractions (1 ml) was determined on a pH meter. Fractions that contained biologic activity were pooled and dialyzed against distilled water to remove ampholytes and referred to as Mono P follicle regulatory protein.

Separation of follicle regulatory protein from Mono P follicle regulatory protein by gel-permeation chromatography. Preparative scale gel-exclusion chromatography was performed with a Spherogel TSK-G3000SWG column fitted to a Waters high-performance liquid chromatography. Buffer D containing 0.02 mol/L sodium phosphate and 0.1 mol/L sodium chloride, pH 7.0, was used as an eluent. Samples (2 to 8 ml) obtained from Mono P chromatography were reduced to approximately 2 ml by ultrafiltration with the use of an Amicon Diaflo system with PM 10 membranes just before column injection. Eluent fractions in the apparent molecular weight range 10,000 to 20,000 daltons were pooled, dialyzed, and lyophilized. Those fractions with follicle regulatory protein activity are referred to as preparative TSK follicle regulatory protein.

Estimations of molecular weight by size-exclusion chromatography on preparative TSK-G3000SWG and analytical TSK-250 columns were performed with the use of gel filtration standards of blue dextran (molecular weight 2,000,000 daltons), bovine serum albumin (molecular weight 67,000 daltons), ovalbumin (molecular weight 45,000 daltons), and ribonuclease A (molecular weight 13,700 daltons).

All determinations of protein concentrations were performed by the method of protein dye binding. Tests for statistical significance were performed with Duncan's new multirange test.

Bioassays of follicle regulatory protein activity

Granulosa cell assays. Porcine granulosa cells (10^5 per well in serum-free medium 199) were incubated for 3 hours (37° C, 5% carbon dioxide, 90% nitrogen) with or without either 200 to 800 μg of orange A–bound material or serial dilutions of a more purified preparation of follicle regulatory protein (total volume = 0.5 ml). Ham's F-10 medium with Hepes buffer (0.5 ml) was added and tubes were centrifuged at 200 × g for 15 minutes. Supernatants were discarded, and cells were resuspended in Ham's F-10 medium with Hepes buffer (0.5 ml), then divided into two aliquots (100 μl each). Androstenedione (30 ng/ml) was added and tubes were incubated in a shaking water bath for 2 hours at 37° C. Normal saline solution (0.8 ml) was added to all tubes, which were then centrifuged at 1000 × g for 15 minutes. Supernatants were decanted, extracted two times with ether, then dried under nitrogen. Samples were resuspended in gel buffer and conversion of androgen was measured by established radioimmunoassay.

Cell-free microsomal aromatase assay. The cell-free microsomal aromatase assay was performed as described and validated previously.[11] Microsomal fractions were prepared from human placentas obtained immediately after delivery. The fresh human placentas were weighed and homogenized with buffer F (0.5 mol/L potassium phosphate and 0.25 mol/L sucrose, pH 7.0) on a Waring Blendor for 1 minute. The homogenate was centrifuged at 800 × g for 30 minutes and the resulting supernatant was centrifuged at 120,000 × g for 1 hour. The precipitate was resuspended in buffer F containing 50% glycerol and stored in the freezer (−60° C).

Test fractions of 0.02 ml were preincubated with 0.3 ml of placental microsome fraction in a 1:60 dilution of stock for 20 minutes at 37° C. A solution (0.1 ml) containing carbon 14–labeled androstenedione (2×10^{-5} mol/L, 52 μCi/mmol), 0.2 mol/L nicotinamide, and 10^{-4} mol/L reduced nicotinamide adenine dinucleotide phosphate in a 1:10 dilution of buffer F was added to the preincubated mixture and incubated for 30 minutes at 37° C, after which the reaction was terminated by ether extraction, 5 ml two times. All incubations were performed in duplicate. Ether extracts were taken to dryness under nitrogen and resuspended in 2 ml of 2,2,4-trimethylpentane (J. T. Baker, Phillipsburg). These solutions were separated by a Celite column with cylohexane/benzene (97:3) to separate androstenedione and testosterone followed by 15% ethyl acetate/2,2,4-trimethylpentane to separate estrogen. The fractions were evaporated to dryness under nitrogen after which 10 ml of scintillation fluid was added for counting.

Conversion rates from androstenedione to estrogen in each fraction were calculated. Inhibitory activities determined by the cell-free aromatase assay were expressed as the percentage of inhibition of conversion rates in sample fractions divided by those in control fractions.[11]

Results

Ammonium sulfate precipitation of porcine follicular fluid. Previously we reported the recovery of follicle regulatory protein as evidenced by inhibition of aromatase in the hypophysectomized immature female rat bioassay from the dye matrex chromatographic

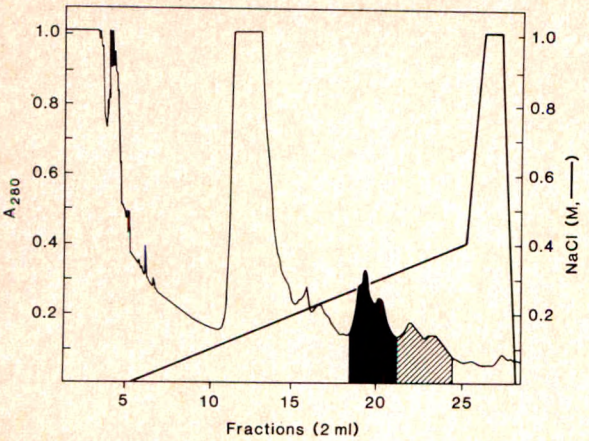


Fig. 1. Ion-exchange elution profile of the dye matrex orange A–bound material developed through a Mono Q column by a sodium chloride gradient under fast protein liquid chromatography conditions. The fractions that contained biologic activity are contained in the *shaded area* (ID_{50} = 120 μg/ml in granulosa cell assay).

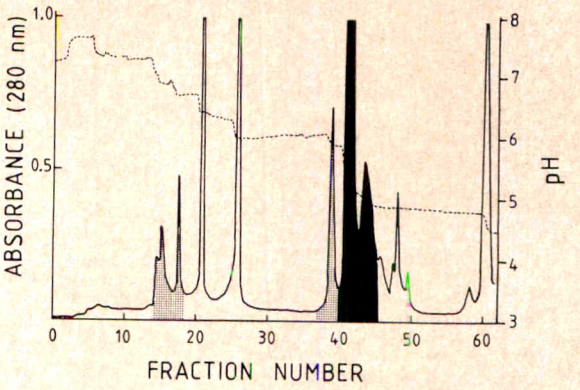


Fig. 2. Hydrogen ion–exchange elution profile of the Mono Q material developed through a Mono P column by a change in pH. The fractions that contained biologic activity are contained in the *shaded areas* (pI = 4.5; ID_{50} = 15 μg/ml in cell-free aromatase assay; ID_{50} = 50 μg/ml in granulosa cell assay).

fraction of the 10% to 55% saturated ammonium sulfate precipitate of porcine follicular fluid.[3] Here we report the relative follicle regulatory protein activities in different fractions of saturated ammonium sulfate–precipitated porcine follicular fluid. After loading of 0% to 35%, 35% to 50%, and 0% to 50% saturated ammonium sulfate onto dye matrex orange A columns, each bound fraction (0% to 35%, 35% to 50%, and 0% to 50% orange A–bound material) was dialyzed and lyophilized. The 50% inhibitory dose (ID_{50}) of each fraction of orange A–bound porcine follicular fluid was determined in the cell*f*ree aromatase assay. The 0% to 50% and 35% to 50% orange A–bound material contained ID_{50}s of approximately 400 μg/ml, whereas 0% to 35% orange A–bound material contained an ID_{50} of 200 μg/ml. Accordingly, the 0% to 35% saturated ammonium sulfate fraction of porcine follicular fluid was used for all further studies reported here.

Separation of follicle regulatory protein from 0% to 35% saturated ammonium sulfate by dye matrex orange A chromatography. The protein fraction that eluted from the matrex gel orange A column before the addition of 0.5 mol/L potassium chloride to elution buffer A (unbound) comes out in two distinct peaks, neither of which exhibited biologic activity in bioassay. One peak absorbing at 280 nm was eluted from the column with 0.5 mol/L potassium chloride, which contained essentially all of the inhibitory activity. The total amount of protein that eluted in this 0.5 mol/L potassium chloride portion was equal to 9% of the 0% to 35% saturated ammonium sulfate fraction of porcine follicular fluid. The ID_{50} of the orange A–bound 0.5 mol/L potassium chloride fraction for the cell-free aromatase assay was 200 μg/ml. The average yield of orange A–bound material was 620 μ/ml. When unbound follicular fluid fractions were recycled through the orange A column, less than 10% of the total activity was recovered, suggesting that the efficiency of the initial pass-through approached 90%.

Separation of follicle regulatory protein from 0% to 35% orange A–bound material by means of ion-exchange chromatography. The Mono Q HR5/5 ion-exchange column developed via fast protein liquid chromatography separated a follicle regulatory protein fraction from 0% to 35% orange A–bound material with an ID_{50} of 30 μg/ml in the cell-free aromatase (Fig. 1) and 120 μg/ml in the granulosa cell assay. With the use of a 0 to 0.4 mol/L sodium chloride gradient, follicle regulatory protein was recovered in a range of 0.28 to 0.32 mol/L with a yield of 10% from 0% to 35% orange A–bound material.

Separation of follicle regulatory protein from mono Q follicle regulatory protein with use of hydrogen ion–exchange chromatography. A typical hydrogen ion–exchange chromatogram of Mono Q follicle regulatory protein eluted through a Mono P column via fast protein liquid chromatography with biologic activity recovered at pH 6.5 and 4.5 *(shaded areas)* is depicted in Fig. 2. From a 16 mg load, biologic activity could be recovered in a relative concentration of 0.02 μg/ml (pH 6.5) and 0.04 μg/ml (pH 4.5). The overall yield was 130 μg (pH 6.5) and 1.1 mg (pH 4.5) from a 16 mg charge of Mono Q follicle regulatory protein. The pH 4.5 follicle regulatory protein has an approximate ID_{50} of 15 μg/ml in the microsomal aromatase assays and an ID_{50} of 50 μg/ml in granulosa cell assay.

Separation of follicle regulatory protein from mono P follicle regulatory protein (pH 5.5) with use of gel-permeation high-performance liquid chromatography. Fig. 3 represents the elution profile from a prep-

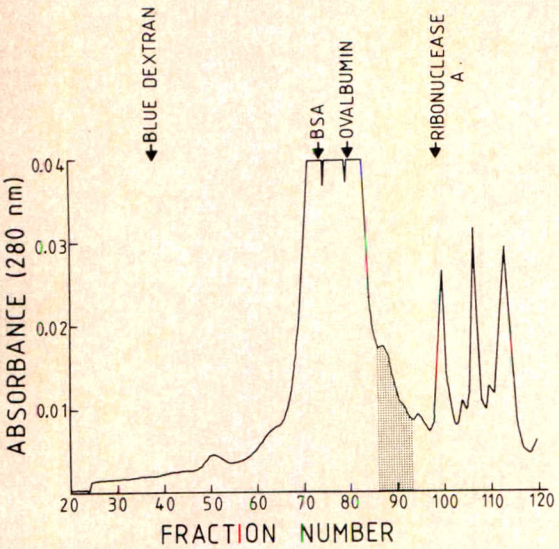


Fig. 3. Elution profile of Mono P, pH 4.5 follicle regulatory protein of porcine follicular fluid recovered from Spherogel preparative TSK-G3000SWG gel permeation column. The *shaded area* corresponds to a molecular weight of 12,000 to 20,000 daltons. A total of 2 ml of pH 4.5 Mono P follicle regulatory protein (2 to 5 mg of protein) was injected per elution at a flow rate of 2.0 ml/min, 300 psi, for 120 minutes. The fraction that contained biologic activity is contained in the *shaded areas* (cell-free aromatase assay: ID_{50} = 1.2 μg/ml; porcine granulosa cell aromatase: ID_{50} = 0.8 μg/ml).

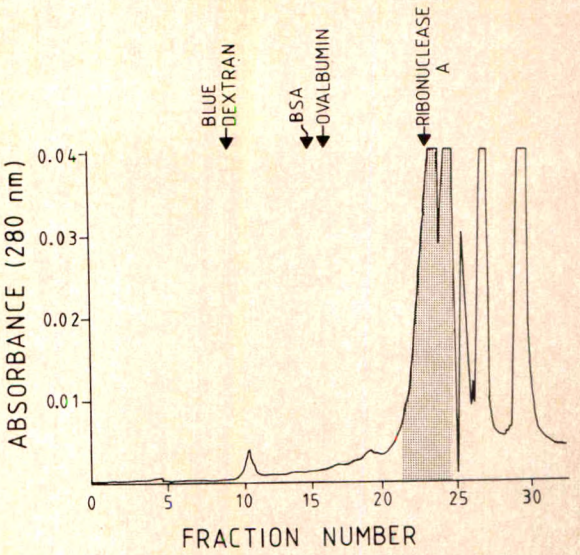


Fig. 4. Chromatography of preparative TSK follicle regulatory protein on analytical TSK-250SW column. A total of 200 μl of preparative TSK follicle regulatory protein was injected per elution. The initial peak (46.03 to 47 minutes) contained aromatase inhibitory activity in the placental cell-free (ID_{50} = 0.030 μg/ml) and porcine granulosa cell (ID_{50} = 0.035 μg/ml) assays.

arative TSK-3000 SW column of Mono P, pH 4.5 follicle regulatory protein. The fraction containing inhibitory activities corresponds to a molecular weight range of 10,000 to 20,000 daltons. The microsomal aromatase inhibitory activity is recovered in 10% of the protein load with an ID_{50} of 5 μg/ml.

The fraction containing follicle regulatory protein on preparative TSK-3000 SW columns was eluted through the analytical TSK-250 column (Fig. 4). The follicle regulatory protein activity was present in fractions corresponding to a molecular weight of 16,000 daltons. The ID_{50} of follicle regulatory protein after analytical TSK-250SW separation was 25 ng/ml for the cell-free aromatase assay and 30 ng/ml for the granulosa cell assay.

Throughout each step, parallelism of follicle regulatory protein was apparent in both the cell-free microsomal (Fig. 5) and granulosa cell (Fig. 6) assays. Follicle regulatory protein was purified about 6666-fold from the orange A–bound fraction of porcine follicular fluid (Table I).

Comment

These data report the relative utilities of biochemical techniques in the purification and assay of follicle regulatory protein wth aromatase inhibition used as the hallmark of activity. Reported here are the requisite steps for purifying follicle regulatory protein from follicular fluid and the relative increases in specific activities and yields from each step. Ledwitz-Rigby et al.[15] confirmed the observations of Kling et al.[3] that follicle regulatory protein inhibits aromatase activity in porcine granulosa cells from medium-sized follicles. Although these observations can be extended to the small follicles of granulosa cells, an effect of follicle regulatory protein on estrogen secretion by cells from large follicles was undetectable. Since reduced aromatase activity in atretic antral follicles is due to a loss of existing aromatase activity rather than a failure to acquire this activity initially, a decrease in aromatase activity is probably an early event in the atretic degeneration of antral follicles. Thus, in follicular atresia, it is the lack of aromatase and not androgen substrate per se that limits estrogen production. The demonstration that follicle regulatory protein acts only on granulosa cells of less mature antral follicles to inhibit aromatase suggests a role for follicle regulatory protein in atresia.

In addition to inhibiting aromatase activity, follicle regulatory protein induces a biphasic effect on progesterone secretion by granulosa cells from sows[16] and rats.[13] At low doses, follicle regulatory protein stimulates progesterone secretion while at higher doses it is inhibitory. When follicle regulatory protein was added to human menopausal gonadotropin-treated granulosa cells, there was a follicle regulatory protein–associated, dose-dependent decrease in progesterone concentrations in culture media and cytosol as well as microsomal

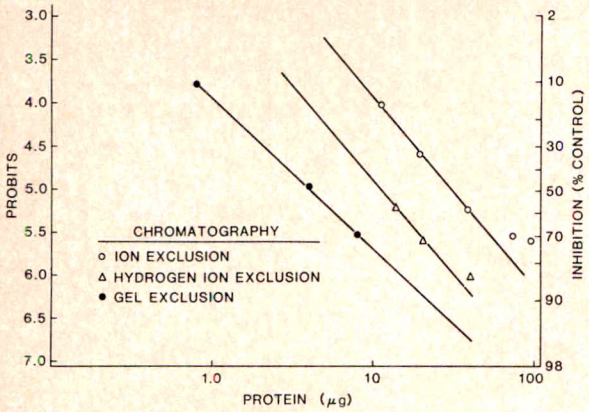


Fig. 5. Inhibition of cell-free placental aromatase activity by each purification step of follicle regulatory protein.

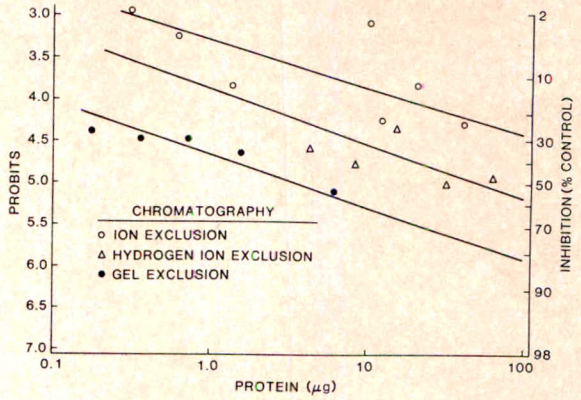


Fig. 6. Inhibition of granulosa cell aromatase activity by each purification step of follicle regulatory protein.

3β-hydroxysteroid dehydrogenase activity. These observations are similar to those of Ledwitz-Rigby et al.,[15] who found that follicle regulatory protein stimulated basal progesterone secretion by porcine granulosa cells with small and medium follicles but not with large follicles. Schreiber and diZerega[13] examined the interactions of follicle regulatory protein and ovine follicle-stimulating hormone on 20α-dihydroprogesterone production in rat granulosa cell cultures. With low doses of ovine follicle-stimulating hormone (6.25 to 37.5 ng/ml), follicle regulatory protein inhibited ovine follicle-stimulating hormone stimulation of 20 α-dihydroprogesterone production. This inhibition by follicle regulatory protein was overcome when ovine follicle-stimulating hormone was increased to 50 ng/ml.

Testosterone appears to sensitize granulosa cells to the action of follicle regulatory protein on steroid secretion.[20] Atretic follicles have been reported to contain higher androgen levels than nonatretic follicles. While androgens have long been thought to contribute to atresia, the exact cause-and-effect relationship between androgens and atresia has remained elusive. Elevated intrafollicular androgen levels may enhance the sensitivity of specific follicles to follicle regulatory protein, causing inhibition of steroidogenesis (especially estrogen production) necessary for follicular growth.

It is unlikely that the effect of follicle regulatory protein on granulosa cell steroidogenesis is due to toxicity of the follicular fluid preparation, since follicle-stimulating hormone is able to overcome follicle regulatory protein inhibition of whole granulosa cell progesterone secretion as well as microsomal 3β-hydroxysteroid dehydrogenase activity, and the effects of follicle regulatory protein on whole porcine,[16] and rat[13] granulosa cell aromatase activity are reversible. Although follicle regulatory protein inhibits aromatization in vitro, it is unlikely that the action of follicle regulatory protein on progesterone secretion simply reflects local reduction in estrogen production, since physiologic concentrations of estradiol suppress progesterone production in human granulosa cells, probably by suppressing 3β-hydroxysteroid dehydrogenase activity. Follicle regulatory protein may have more than one site of action in the cell, or it may contain more than one active element.

Since follicle regulatory protein is an effective inhibitor of aromatase activity, the possibility remains that the buildup of endogenous intracellular androgen by the follicle regulatory protein–mediated inhibition of aromatase may alter 3β-hydroxysteroid dehydrogenase activity. Aromatizable androgens inhibit follicle-stimulating hormone–stimulated progesterone synthesis in porcine granulosa cells at a step distal to cyclic adenosine monophosphate formation. Further, aromatizable androgens markedly increase pregnenolone accumulation in follicle-stimulating hormone–treated cultures. Previous studies also demonstrated that follicle regulatory protein has no apparent effect on follicle-stimulating hormone receptor binding in granulosa cells.[3, 8] Therefore, the stimulatory effect of follicle regulatory protein is probably not a direct function of the follicle-stimulating hormone receptor. Recent studies carried out on follicle-stimulating hormone–augmented adenylate cyclase activity indicate that follicle regulatory protein inhibits follicle-stimulating hormone induction of adenylate cyclase.[9] Interestingly, reports describing the effects of follicle regulatory protein on human placental microsome preparations suggest that follicle regulatory protein noncompetitively inhibits aromatase[10] and 3β-hydroxysteroid dehydrogenase activities.[12]

Steroidogenic enzymes in granulosa cells appear to have follicle-stimulating hormone–dependent differential sensitivities to follicle regulatory protein in vitro. Follicle regulatory protein in concentrations of 50 to 200 μg/ml inhibits aromatase activity in granulosa

Table I. Purification of a follicular regulatory protein from porcine follicular fluid with the use of cell-free placental aromatase and granulosa cell assays

Stage of purification	*Protein added (mg)*	*Protein recovery (%)*	*Active mass (mg)*	*Yield (mg/100 ml)*	*Activity*		*Purification (fold)*
					Granulosa cell assay (per ml)	*Microsome assay (per ml)*	
Porcine follicular fluid	—	—	—	8800	—	—	—
0% to 35% Saturated ammonium sulfate	88000	90	19890	1989	—	—	—
Orange A	4689	98	430	171	ID_{50} 300-600 μg	ID_{50} 200 μg	1
Ion exchange—Mono Q	200	98	21	18	ID_{50} 120 μg	ID_{50} 30 μg	7
Hydrogen ion exchange—Mono P	20	60	3.4	3.3	ID_{50} 50 μg	ID_{50} 15 μg	13
Preparative gel exclusion	2.	90	0.06	0.08	ID_{50} 800 ng	ID_{50} 1.2 μg	167
Analytical gel exclusion	0.015	90	0.005	0.08	ID_{50} 25 ng	ID_{50} 30 ng	6666

cells[13, 16] while stimulating 3β-hydroxysteroid dehydrogenase activity. When the concentration of orange A–bound follicle regulatory protein is further increased to 500 μg/ml, both aromatase and 3β-hydroxysteroid dehydrogenase activities are inhibited. Granulosa cells exposed to follicle-stimulating hormone alone produce more estrogen and less progesterone. However, when granulosa cells are exposed to follicle-stimulating hormone in the presence of follicle regulatory protein, more progesterone and less estrogen are produced. These observations suggest that follicle regulatory protein alters the dose-response relationship between follicle-stimulating hormone and 3β-hydroxysteroid dehydrogenase activity in granulosa cells. Thus follicle regulatory protein may play a role in modulating the relative secretion of progesterone and estrogen by granulosa cells in response to follicle-stimulating hormone stimulation.

Kinetic analysis of follicle regulatory protein inhibition of aromatase and 3β-hydroxysteroid dehydrogenase activities is suggestive of noncompetitive inhibition.[10, 12] The inhibitory effects of follicle regulatory protein on aromatase and 3β-hydroxysteroid dehydrogenase activities in placental microsomes were associated with a decrease in the apparent maximum velocity without a detectable change in the apparent Michaelis constant of the enzyme. Thus the possibility that follicle regulatory protein treatment decreases enzyme activity by decreasing the affinity of the enzyme for its substrate is unlikely. The apparent inhibition constant for follicle regulatory protein–mediated inhibition of 3β-hydroxysteroid dehydrogenase was 10^{-9} mol/L, at least an order of magnitude more sensitive than the inhibition constant for aromatase inhibition, 10^{-7} mol/L. This derived inhibition constant for follicle regulatory protein is within the biologic concentration of follicle regulatory protein in follicular fluid, assuming that the molecular weight of follicle regulatory protein is 16,000. Schreiber and diZerega[13] and Battin and diZerega,[16] using porcine and rat granulosa cells, also found that 3β-hydroxysteroid dehydrogenase activity was more sensitive than aromatase activity to follicle regulatory protein inhibition.

Most of the effects of follicle regulatory protein on granulosa cell activities may reflect an interplay between the systemic endocrine and local paracrine systems. Since follicle regulatory protein alters ovarian steroidogenesis, it may also have an influence on maintenance of the dominant ovarian follicle. Many investigators have hypothesized that a timely induction or activation of the granulosa cell aromatase system and the concomitant changes in the intrafollicular sex steroid profiles are major determinants of the dominant follicle during its selection early in the ovarian cycle. Once a chosen follicle is producing a significant amount of estrogen, it would then have the capacity to produce more estrogen than neighboring follicles and the selection of the dominant follicle would be assured. In contrast, follicles destined to undergo atresia are not able to produce sufficient estrogen. Thus the developing granulosa cell, through production of paracrine regulatory substances such as follicle regulatory protein, is capable of autoregulating the steroid production of its own and other follicles and thus the subsequent course of folliculogenesis.

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A Standard



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(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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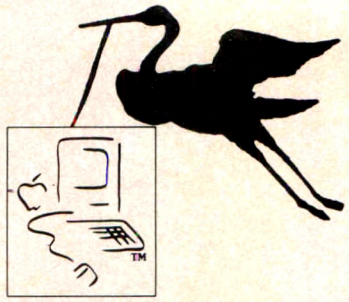
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Registration/Breakfast

8:00-10:00 AM

I. Introduction
- A. course goals and objectives
- B. orientation to the equipment *("hands-on")*
- C. overview of course content

II. Word Processing: "Hands-on experience with some practical applications"
- A. introduction to word processing
- B. using MacWrite™ *("hands-on")*
  1. the absolute basics
  2. practical examples
     - a. general: referral letters, manuscripts, personal letters
     - b. special: slide production, newsletters, posters, flyers
  3. adding the "bells and whistles"
     - a. checking your spelling
     - b. customized "form letters"
- C. other word processing programs
  1. Microsoft Word™
  2. specialized programs: ThinkTank™, Page Maker™, etc.
  3. IBM PC word processors: comparisons and compatibility

10:15 AM-12:30 PM

III. Educational Programs:
for your patients, staff, family and you
- A. an animated example of computerized medical education *("hands-on")*
- B. family education: pre-school, school age, college prep, etc.
- C. video disk systems for personal and medical education
- D. patient education: VD, prenatal, contraception, pre-op
- E. on line education: dial-up data bases via telephone lines

SUNDAY, AUGUST 10, 1986

7:00-7:30 AM
Registration / Breakfast

7:30-9:15 AM

IV. Data Base Management:
computerized filing systems
- A. introduction to data base
- B. using Microsoft File™ *("hands-on")*
  1. how to use a data base
  2. how to set up your own data base
  3. additional examples: OB-log book, case lists, practice profile
- C. "heavy-duty" data base applications
  1. relational data bases: Helix™, Omnis 3™, and Interlace™
  2. data import/export: mainframe computers, IBM micro's, word processing programs, other programs

9:30 - 11:15 AM

V. Electronic Spreadsheets:
computerized calculations and projections
- A. definitions and basic concepts
- B. impact of spreadsheets on medical practice
  1. insurance companies, politicians, and medical organizations
  2. diversity of applications: USG, statistics, financial, medical
- C. Using Microsoft Excel™ *("hands-on")*
  1. getting oriented to the spreadsheet
  2. practical examples: EFW by USG, tax calculations, etc.
  3. adding the finishing touches
     - a. fancy formatting
     - b. graphs and charts
- D. Sophisticated examples of spreadsheet applications *("hands-on")*

11:30 – 12:30 PM

VI. Conclusions
- A. Getting your first computer system
  1. how to select and purchase the equipment
  2. software (programs): What do you really need?
- B. What can you, should you, and will you do with your computer?
- C. Microcomputers vs. big computers: the right tool for the right job
- D. The future of medical microcomputing

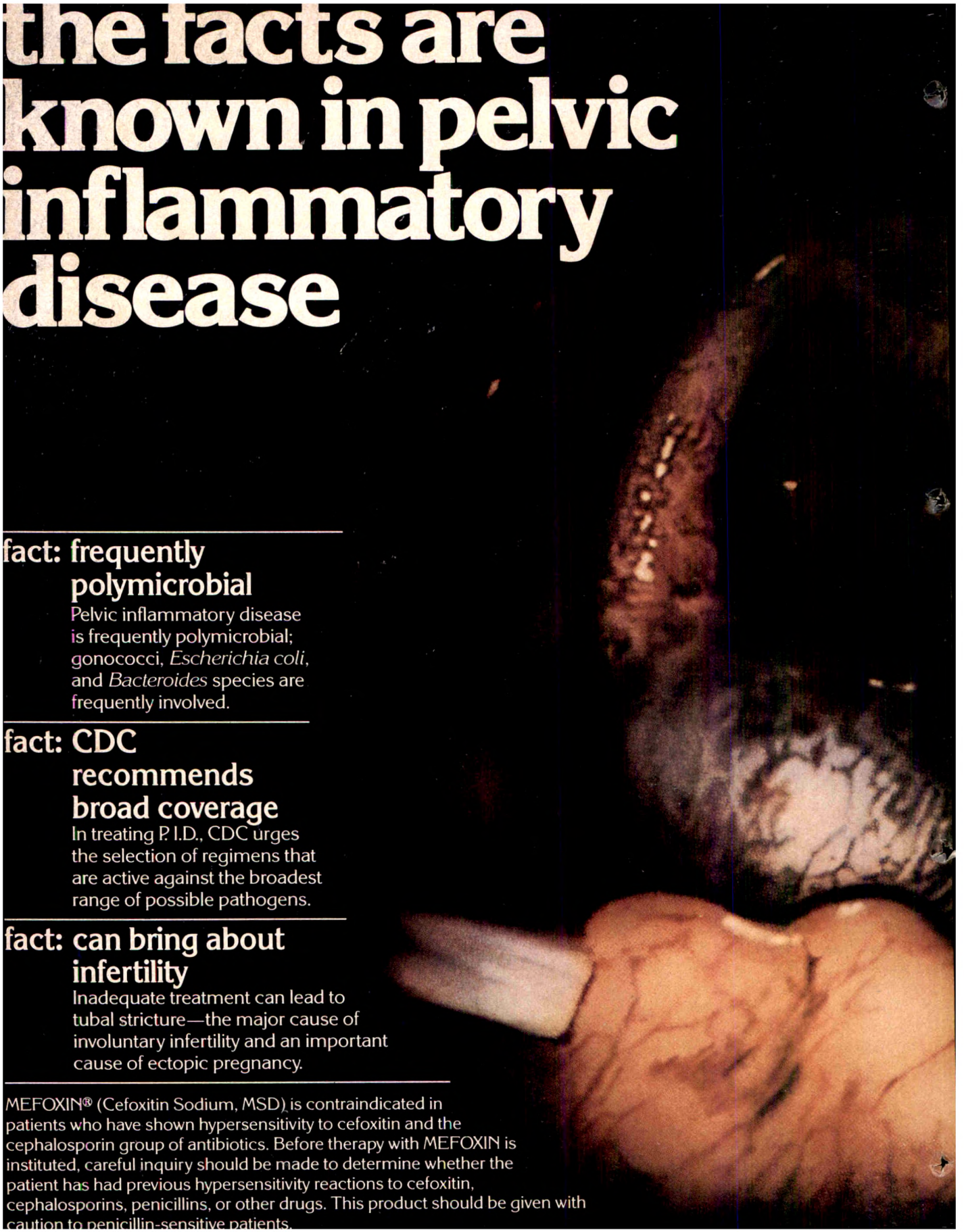
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FRONTIER HOTEL

For Seminar Information Write:

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the facts are known in pelvic inflammatory disease



fact: frequently polymicrobial

Pelvic inflammatory disease is frequently polymicrobial; gonococci, *Escherichia coli*, and *Bacteroides* species are frequently involved.

fact: CDC recommends broad coverage

In treating P. I.D., CDC urges the selection of regimens that are active against the broadest range of possible pathogens.

fact: can bring about infertility

Inadequate treatment can lead to tubal stricture—the major cause of involuntary infertility and an important cause of ectopic pregnancy.

MEFOXIN® (Cefoxitin Sodium, MSD) is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients.



Unruptured ampullary ectopic pregnancy.

and in the treatment of P.I.D.†

the facts still favor Mefoxin® IV/IM

PROVEN* FOR 7 SEVEN YEARS

(Cefoxitin Sodium | MSD)

fact: consistent spectrum of activity**

MEFOXIN has maintained a high level of activity against common pathogens in P.I.D. during 7 years of extensive clinical use.

fact: documented clinical efficacy

CDC has recommended that MEFOXIN, in combination with doxycycline, be one of the regimens for the treatment of P.I.D.

fact: safety comparable to cephalothin

MEFOXIN is generally well tolerated. MEFOXIN does not contain the MTT side chain which has been associated with hypoprothrombinemia and, in some cases, clinical bleeding.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use.

†MEFOXIN is indicated for the treatment of pelvic inflammatory disease caused by *Escherichia coli*, *Neisseria gonorrhoeae* (penicillinase- and non-penicillinase-producing), *Bacteroides* species including the *Bacteroides fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and Group B streptococci. MEFOXIN is not indicated for treatment of pelvic inflammatory disease where *Chlamydia trachomatis* is present or suspected.

*proven for treatment of, and prophylaxis against, infections due to indicated organisms

***In vitro* activity does not necessarily imply *in vivo* effectiveness.

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For a Brief Summary of Prescribing Information, please see following page.

Mefoxin® IV/IM (Cefoxitin Sodium | MSD)

Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ‖ reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk; Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

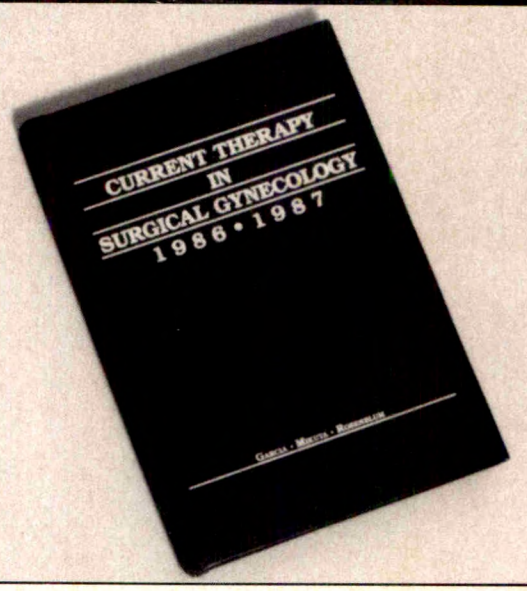
How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

‖ Registered trademark of Ames Company, Division of Miles Laboratories, Inc.

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Further studies on genital mycoplasms in intra-amniotic infection: Blood cultures and serologic response

Ronald S. Gibbs, M.D., Gail H. Cassell, Ph.D., Jerry K. Davis, D.V.M., Ph.D., and P. J. St. Clair, B.A.

San Antonio, Texas, and Birmingham, Alabama

Mycoplasma hominis is recovered significantly more often in amniotic fluid of women with intra-amniotic infection than in matched control women, but *Ureaplasma urealyticum* is found in 50% of amniotic fluid samples of both groups. To gain further understanding, we performed blood cultures for genital mycoplasmas and measured serologic responses by a micro enzyme-linked immunosorbent assay method in women with intra-amniotic infection and in control subjects. In blood cultures of 81 women with intra-amniotic infection, *M. hominis* was isolated in two (2.5%) and *U. urealyticum* in 11 (13.6%). In 44 control blood cultures, *M. hominis* was not isolated, and *U. urealyticum* was recovered in eight (18.2%). These differences were not significant. Serologic response was determined in 86 patients. Rise in antibody to *M. hominis* was significantly more common in women with intra-amniotic infection and *M. hominis* in the amniotic fluid than in either women with intra-amniotic infection or control patients without *M. hominis*. For *U. urealyticum* antibody response was significantly more common in the intra-amniotic infection group than in control subjects, but there was no association between antibody response and isolation of this organism in amniotic fluid. When *M. hominis* was found in amniotic fluid or maternal blood, patients were nearly always symptomatic. The high likelihood of serologic response in these cases supports a pathogenic role of *M. hominis* in intra-amniotic infection. The role of *U. urealyticum* remains unclear. (Am J Obstet Gynecol 1986;154:717-26.)

Key words: Blood cultures, enzyme-linked immunosorbent assay, genital mycoplasmas, intra-amniotic infection, serologic responses

Genital mycoplasmas are found with great frequency in the vagina and have been implicated in the pathogenesis of low birth weight infants, postpartum fever, and histologic chorioamnionitis.[1a, 1b] In 1983, we reported on the relationship of clinically evident intra-amniotic infection and genital mycoplasmas in amniotic fluid cultures.[2] *Mycoplasma hominis* was found significantly more often in the amniotic fluid of patients with intra-amniotic infection than in amniotic fluid of matched, asymptomatic control subjects (36% versus 8%, $p < 0.001$). However, in amniotic fluid of cases of intra-amniotic infection, *M. hominis* was usually (83% of cases) found with $>10^2$ colony-forming units per milliliter of high-virulence bacteria. It has thus been unresolved whether *M. hominis* plays a causal role in intra-amniotic infection. *Ureaplasma urealyticum* was present in 50% of amniotic fluid samples from both women with intra-amniotic infection and asymptomatic control women. Thus, on the basis of qualitative cultures, *U. urealyticum* was not associated with intra-amniotic infection.

To gain a further understanding of the role of genital mycoplasmas in intra-amniotic infection, we performed blood cultures for these microbes and also measured serologic responses in patients with intra-amniotic infection and in asymptomatic control women.

Methods

Specimens for this study were collected from patients delivered between January, 1982, and April, 1985, at Medical Center Hospital, San Antonio, Texas. They were mutually exclusive of patients reported previously.[2] The population consisted predominantly of indigent women of Mexican-American extraction. As in previous studies, intra-amniotic infection was defined on the basis of an oral temperature of >100° F (37.8° C) plus two or more of the following: maternal tachycardia (>100 bpm) and fetal tachycardia (>160 bpm), uterine tenderness, foul odor, or leukocytosis (>15,000/mm^3). Patients were considered asymptomatic if they had no temperature >99.8° F (37.8° C) and

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no other signs of infection at any time in labor or the puerperium. Women who developed clinically evident endometritis, transient low-grade fever, or other infections were excluded. None of the asymptomatic patients received either prophylactic or therapeutic antibiotics.

Culture technique. From each of these patients, an amniotic fluid sample was collected through an intrauterine pressure catheter that had been inserted for standard obstetric indications. In women with intraamniotic infection, the collection of amniotic fluid preceded antibiotic therapy. Samples were collected from control women who had ruptured membranes >10 to 12 hours. The first 7 ml of amniotic fluid was discarded, and the sample was then transported in a capped syringe and cultured within 60 minutes for aerobes, anaerobes, *M. hominis*, and *U. urealyticum*. For most of the study, cultures of genital mycoplasmas from amniotic fluid were performed by use of a modification of the technique described by Kenny.[3] A tube of urea broth was inoculated with 0.2 ml of amniotic fluid, incubated at 37° C, and observed daily for a color change indicating an increase in pH. Immediately on detection of such a color change, a 0.1 ml aliquot of the broth was transferred onto MES [2-(N-morpholino) ethane sulfonic acid] agar (Sigma Chemical Co, St. Louis, Missouri) and into new broth. The MES agar was then placed in an atmosphere of 5% carbon dioxide:95% nitrogen and incubated, and the new urea broth was incubated aerobically at 37° C. When a color change occurred in the new broth, the MES agar was observed for the presence of genital mycoplasmas. Stains and colony morphologic features were used for identification. Colonies of *U. urealyticum* turn brown with urea test reagent, and colonies of *M. hominis* stain blue with Dienes stain (Difco Laboratories, Detroit, Michigan).

For the last third of the study, amniotic fluid was inoculated onto a Shepard A7B agar plate and broth media, and these were incubated at 37° C. The agar plates were examined 3 days later. Identification of colonies of genital mycoplasmas was made by colonial morphologic features as above. The broth media were incubated as backup in case the primary agar plate did not recover the organism. Isolation rates with the use of these media were comparable to the rates with the technique of Kenny.

The amniotic fluid was also cultured quantitatively for bacteria on a variety of aerobic and anaerobic media.[4]

For culturing of maternal blood, 1 ml was collected aseptically into 9 ml of combination broth and incubated at 37° C for up to 8 days or until a color change indicating a rise in pH occurred.[5] As soon as a color change from orange-red to deep red occurred, the broth was subcultured onto urea agar, arginine agar, and a new combination broth and incubated for 48 hours anaerobically at 37° C. The agar plates were inspected with a stereoscopic microscope at 48 hours. Genital mycoplasmas were identified by colonial morphologic features, as described above. If no color change occurred in the original combination broth, then a blind subculture was taken at 48 hours and processed as above. The maternal blood was also cultured for aerobes and anaerobes, with the use of 5 ml of blood in 70 ml of tryptic soy broth, with 0.05% sodium polyanetholsulfonate. Standard isolation and identification techniques were used.

Samples of infants' blood were collected, when clinically indicated, and cultured for bacteria by standard techniques. As recommended by Galando and McCormack,[5] a blind subculture was taken on the seventh day and processed for genital mycoplasmas.

Serologic techniques. Peripheral maternal blood was collected within 2 days of delivery and again approximately 4 to 6 weeks post partum. Sera were stored at −70° C until assayed. IgG antibodies were detected by a micro enzyme-linked immunosorbent assay. Samples were assayed in blinded fashion by a modification of techniques previously reported.[6, 7] Antigen preparations of each microorganism in logarithmic growth (12 to 20 hours, 10^5 to 10^8 cfu/ml, dependent on mycoplasma strain) were diluted in broth (1:5 to 1:20) and harvested by centrifugation at 10,000 × g for 30 minutes at 4° C after a color change (12 to 20 hours). The pellet was resuspended and washed three times with sterile phosphate-buffered saline, pH 7.3. Bacterial contamination was monitored by inoculation of tryptic soy and blood agar plates. The washed pellet of cells was resuspended in phosphate-buffered saline (5 mg of protein per milliliter) and lysed by addition of 0.05 mol/L carbonate-bicarbonate buffer, pH 10.0, at 37° C. The protein content of each lysate was determined, and all antigens were stored at −70° C.

Antigen lysates for *M. hominis* were diluted in 0.1 mol/L sodium carbonate buffer (pH 9.6) to a concentration of 50 μg/ml; 100 μl of antigen was added to each well (Linbro 96 well microliter E.I.A. plates; Flow Laboratories) and plates were incubated at 37° C for 2 hours. These plates were washed with phosphate-buffered saline (pH 7.3) and blocked overnight with 2% bovine serum albumin in phosphate-buffered saline with 10% horse serum. After removal of the blocking agent, 100 μl of a 1:200 serum dilution was added to each well in duplicate and incubated for 2 hours at 37° C. A background control in which the serum diluent (blocking agent) was substituted for serum was used in addition to positive and negative reference standards. After incubation with serum, the plates were washed

Table I. Characteristics of patients from whom blood cultures were collected

Characteristic	*Intra-amniotic infection (n = 81)*	*Asymptomatic (n = 44)*	*p*
Age* (yr)	22.2 ± 5.7	21.9 ± 6.1	NS
Nulliparity (n and %)	63 (77.8%)	27 (61.4%)	NS
Gestational age* (wk)	39.5 ± 2.5	39.1 ± 2.0	NS
Duration of labor* (hr)	12.6 ± 5.3	8.6 ± 4.0	<0.0001
Interval, rupture of membranes to delivery* (hr)	30.0 ± 27.0	17.5 ± 17.9	0.006
Duration of internal monitoring* (hr)	13.3 ± 8.2	7.8 ± 5.4	0.0001
Interval, collection to delivery* (hr)	5.0 ± 3.6	4.0 ± 3.2	NS
Maximum temperature in labor* (°F)	101.1 ± 0.7	98.7 ± 0.4	<0.0001
Cesarean delivery (%)	34 (42.0)	0	<0.0001
Birth weight* (gm)	3305 ± 632	3225 ± 459	NS

*Data expressed as mean ± SD.

six times, and 100 μl of anti-immunoglobulin conjugate (antiserum to human γ-chain, Kirkegaard and Perry Laboratories) was added. Plates were incubated for 3 hours at 37° C and washed six times with phosphate-buffered saline. Finally, 100 μl of alkaline phosphatase substrate (Sigma Chemical Co.) in 0.05 mol/L sodium carbonate buffer with 0.001 mol/L magnesium chloride (pH 9.8) was added to each well and plates were incubated at 37° C for 45 minutes. The absorbance was determined at 400 nm.

Antigen preparation for the *U. urealyticum* enzyme-linked immunosorbent assay was as described for the *M. hominis* assay. However, some of the other steps differed slightly. The plates were coated with 100 μl of a 50 μg/ml preparation and were stored overnight at 4° C. The plates were then washed with phosphate-buffered saline (pH 7.3) six times and blocked for 2 hours at 37° C with 2% bovine serum albumin in phosphate-buffered saline with 10% fetal calf serum. After removal of the blocking agent, patient sera were diluted 1:200 and 100 μl of this dilution was added to each well in duplicate. Incubation was 2 hours at 37° C. Plates were then washed with phosphate-buffered saline six times and the conjugate was allowed to incubate overnight (18 hours) at 4° C. Plates were washed and alkaline phosphatase substrate (Sigma Chemical Co.) was added to each well. After a 30-minute incubation, plates were read at 400 nm.

Optimal incubation periods and concentrations of antigen, conjugate, and serum were determined by checkerboard titration for all assays. A sample was considered "positive" if its value of absorbance at 400 nm (A_{400}) was greater than the mean normal serum standard value (from nonpregnant adults in Birmingham) +2 SD. A sample was "weakly reactive" if it was twice the mean normal serum standard value but <2 SD above the mean. Seroconversion was defined as change from negative to positive, or negative to weakly reactive, or weakly reactive to positive.

Serologic response was measured in three ways: (1) by comparing number (percent) with seroconversion, (2) by comparing number (percent) with increases in $A_{400} > 0.200$ optical density units, and (3) by comparing mean difference in A_{400} between convalescent and acute sera.

Statistical analysis was by χ^2 or Fisher's exact test (where appropriate) for discrete data and analysis of variance for continuous data.

Results

Blood cultures for genital mycoplasmas and bacteria were collected during labor from 81 patients who had intra-amniotic infection and from 75 patients who were asymptomatic. Of the latter, six subsequently developed endometritis and 25 developed transient puerperal fever; these 31 patients were excluded. The other 44 remained asymptomatic. Characteristics of the intra-amniotic infection and asymptomatic groups are shown in Table I. The groups were similar in mean age, parity, and gestational age. Although both groups had long intervals from rupture of the membranes to delivery, this interval was significantly longer in the intra-amniotic infection group.

Blood and amniotic fluid culture results are shown in Table II. Maternal blood cultures revealed *M. hominis* in two of 81 (2.5%) of all patients with intra-amniotic infection and in two of 33 (6.1%) patients with both intra-amniotic infection and amniotic fluid positive for *M. hominis*. *U. urealyticum* was found more commonly in the bloodstream of both intra-amniotic infection and control groups, but there was no significant difference in ureaplasmemia rates. When genital mycoplasmas were found in the blood, the corresponding isolate was found in amniotic fluid in 18 (86%) of 21 cases. Mycoplasmemia was not detected in any of 60 neonatal blood cultures from infants born to women with intra-amniotic infection. Blood cultures were not collected in asymptomatic infants of control women.

Table II. Blood and amniotic fluid culture results from 81 patients with intra-amniotic infection and 44 asymptomatic control subjects

Source	*Intra-amniotic infection*		*Asymptomatic*		*p*
	n	%	*n*	%	
Maternal blood					
M. hominis	2/81	2.5	0/44	0	NS
U. urealyticum	11/81	13.6	8/44	18.2	NS
Bacteria*	4/80	5.0	0/44	0	NS
Amniotic fluid					
M. hominis	33/81	40.7	5/44	11.3	0.001
U. urealyticum	44/81	54.3	21/44	47.7	NS
High-virulence bacteria ($\geq 10^2$ cfu/ml)	55/81	67.9	16/44	36.4	0.001
Neonatal blood					
M. hominis	0/60	0	0/0		
U. urealyticum	0/60	0	0/0		
Bacteria†	6/77	7.8	0/2		

*Maternal isolates: group B streptococci, two; *Escherichia coli,* one; *Staphylococcus aureus,* one.
†Neonatal isolates: α-streptococci, one; group B streptococci, one; .
Hemophilus influenzae, one; *E. coli,* one; unidentified gram-positive cocci, one.

Bacteremia was identified in four (5%) women with intra-amniotic infection, no (0%) asymptomatic women, and six (7.8%) of 77 neonates born after intra-amniotic infection.

Paired sera were available from 52 patients with intra-amniotic infection and from 34 control subjects. (In two women with intra-amniotic infection and one control women, amniotic fluid cultures for mycoplasmas were contaminated.) Characteristics of women in this part of the study are shown in Table III. There were no significant differences in age, parity, gestational age, or interval from amniotic fluid collection to delivery, but the intra-amniotic infection group had a significantly longer mean labor and longer mean interval from rupture of the membranes to delivery. Within the intra-amniotic infection group, there were no significant differences in characteristics between patients with positive and negative amniotic fluid cultures for *M. hominis* or for *U. urealyticum.* Similarly, there were no significant differences in characteristics within the subgroups of asymptomatic patients. Thirty-five women participated in both the blood culture and serologic studies.

The mean (± SD) interval between collection of acute and convalescent sera was 5.4 ± 1.8 weeks, and there were no significant differences in collection intervals among the subgroups shown in Tables IVA and IVB. In the 20 patients with intra-amniotic infection and with *M. hominis* in amniotic fluid, seroconversion occurred in 14 of 20 (70%), and a rise >0.2 optical density units occurred in six (30%). Seventeen (85%) of these patients showed either seroconversion or a rise >0.2 optical density units. Compared with either intra-amniotic infection or control patients without *M. hominis* in amniotic fluid, these serologic changes were significantly greater. Serologic response did not correlate with route of delivery. Of the 14 patients with intra-amniotic infection, *M. hominis* in the amniotic fluid, and seroconversion, six (43%) were delivered by cesarean section. Overall, the cesarean section rate in the intra-amniotic infection group was 46.2% (24 of 52). All IgG values against *U. urealyticum* were positive, but rises >0.2 optical density units in IgG antibodies against *U. urealyticum* occurred significantly more often in the intra-amniotic infection group than in control subjects ($p = 0.007$). However, within the intra-amniotic infection group, there was no association between these rises and isolation of *U. urealyticum* in amniotic fluid. Analysis of serologic changes by mean difference between convalescent and acute levels also led to significant differences.

Four patients whose blood contained *U. urealyticum* had serologic studies performed. Of the two with intra-amniotic infection, one had an IgG rise of >0.2 optical density units. This proportion was similar to that of all patients with intra-amniotic infection. Two patients with ureaplasmemia were asymptomatic, and neither had a rise >0.2. Two patients whose blood contained *M. hominis* had serologic studies. Both had intra-amniotic infection, and both showed IgG rises >0.2.

Comment

M. hominis and *U. urealyticum* are found in approximately 35% to 50% and 50% to 70% of vaginal cultures, respectively.[1] Previous investigators have implicated these microbes in low birth weight infants, postpartum fever, and histologic chorioamnionitis. We have been interested in the role of these organisms in clinically

Table III. Characteristics of patients in whom serologic studies were performed

Characteristics	*Intra-amniotic infection (n = 52)*	*Asymptomatic (n = 34)*	*p*
Age (yr)	22.1 ± 5.7	22.9 ± 6.2	NS
Nulliparity (n and %)	41 (78.8%)	21 (61.8%)	NS
Gestational age* (wk)	39.7 ± 2.9	39.7 ± 1.7	NS
Duration of labor* (hr)	13.6 ± 5.5	9.6 ± 4.1	0.0004
Interval, rupture of membranes to delivery* (hr)	32.6 ± 16.4	16.6 ± 15.8	0.002
Internal monitoring, duration* (hr)	14.2 ± 8.0	8.7 ± 4.6	0.0006
Interval, collection to delivery* (hr)	6.4 ± 4.7	4.4 ± 3.2	0.03
Maximum temperature in labor* (°F)	101.0 ± 0.9	98.6 ± 0.4	<0.0001
Cesarean section (n and %)	24 (46.2%)	0 (0%)	<0.0001
Birth weight* (gm)	3478 ± 694	3331 ± 494	NS
Amniotic fluid			
M. hominis present (n and %)	20/50 (40.0%)	3/33 (9.1%)	0.002
U. urealyticum present (n and %)	22/50 (44.0%)	14/33 (42.4%)	NS
High-virulence bacteria ($\geq 10^2$ cfu/ml) (n and %)	31/50 (62.0%)	11/33 (33.3%)	0.011

*Data expressed as mean ± SD.

evident intra-amniotic infection and found *M. hominis* significantly more often in the amniotic fluid of 52 women with intra-amniotic infection than in fluid of 52 matched, asymptomatic control subjects (36% versus 8%, $p < 0.001$).[2] *U. urealyticum* was found in 50% of amniotic fluid cultures in both the intra-amniotic infection and control groups (NS). The amniotic fluid culture results in this series are very similar.

However, these data do not provide conclusive evidence of either a pathogenic role for *M. hominis* or the lack of a pathogenic role for *U. urealyticum* in intra-amniotic infection. Indeed, when *M. hominis* was found in amniotic fluid of intra-amniotic infection patients, it was usually present (15 of 18 cases, 83%) with $>10^2$ cfu/ml of high-virulence bacteria.[2] Accordingly, it is possible that *M. hominis* simply becomes more prevalent in amniotic fluid as a consequence of intra-amniotic infection rather than as its cause. For *U. urealyticum*, it might be that in intra-amniotic infection this organism is present in amniotic fluid in large numbers, whereas in asymptomatic patients it is present in small numbers. Because quantitative cultures of amniotic fluid for genital mycoplasmas were not done, we cannot resolve this point.

To understand further the role of genital mycoplasmas in intra-amniotic infection, we looked for mycoplasmemia and for serologic responses in patients with intra-amniotic infection and in asymptomatic control women.

Presence of an organism in the bloodstream is often accepted as strong evidence of its pathogenicity. However, microorganisms introduced during collection or laboratory processing are occasionally found as contaminants in blood cultures, on the one hand, and bacteremia occurs asymptomatically in at least 2% of puerperal women, on the other hand. Accordingly, blood culture results must be interpreted in view of organisms present at the infected site (in the case of intra-amniotic infection, the amniotic fluid) and in view of the clinical course.

No previous work has systematically assessed mycoplasmemia in intra-amniotic infection (or clinical chorioamnionitis), but several reports have investigated bloodstream invasion by mycoplasmas in the puerperium. Like bacteria found in the lower genital tract, mycoplasmas have been found at least transiently in the bloodstream immediately after delivery. In 1975, McCormack et al.[8] recovered genital mycoplasmas from 26 of 327 (8%) asymptomatic women within a few minutes after vaginal delivery (*M. hominis* was recovered from 10, *U. urealyticum* from 15, and both from one). These episodes were transient even without specific antibiotic therapy. Of 21 women with mycoplasmemia, 20 (95%) had negative blood cultures upon repeat testing one or more days later. *M. hominis* was isolated from the vaginal culture of nine of 10 (90%) women with this organism in the blood, and *U. urealyticum* was found in the vaginal culture of 10 of 13 (77%) women with ureaplasmemia. Fever after delivery developed in five of 10 (50%) women whose blood contained *M. hominis*, six of 15 (40%) women whose blood contained *U. urealyticum*, and 90 of 301 (30.9%) women whose blood did not contain mycoplasmas (NS). Thus isolation of genital mycoplasmas in blood immediately after delivery was not significantly associated with puerperal fever. Interestingly, four of six women with *M. hominis* in the bloodstream showed a significant rise in mycoplasmacidal antibody titer to homologous isolates whereas none of seven with *U. urealyticum* in the bloodstream showed a serologic response.

There have also been many reports of *M. hominis* in the blood of women with postpartum fever (>24

Table IVA. Serologic and amniotic fluid culture results for *M. hominis* in 50 patients with intra-amniotic infection and 33 asymptomatic control subjects

Antibody response to M. hominis	*Group*				*p*
	Intra-amniotic infection, M. hominis*		*Asymptomatic,* M. hominis*		
	Positive (n = 20)	*Negative (n = 30)*	*Positive (n = 3)*	*Negative (n = 30)*	
Seroversion (n and %)	14 (70%)	9 (30%)	2 (67%)	6 (20%)	0.003
Increase in optical density ≥ 0.2 units (n and %)	6 (30%)	2 (7%)	1 (33%)	0 (0%)	0.035†
Either seroconversion or increase in optical density ≥ 0.2 units (n and %)	17 (85%)	9 (30%)	2 (67%)	6 (20%)	0.001
Acute optical density (mean ± SD)	0.164 ± 0.061	0.142 ± 0.068	0.186 ± 0.100	0.164 ± 0.078	NS
Change in optical density‡ (mean ± SD)	0.180 ± 0.213	0.066 ± 0.123	0.164 ± 0.153	0.036 ± 0.037	0.005

*Results of amniotic fluid cultures.

†p Value is for comparison of intra-amniotic infection groups. Difference in asymptomatic groups is not significant.

‡Change equals convalescent minus acute.

hours after delivery). Since 1968, 25 cases have appeared.[9-11]* Available data reveal a mean maximum temperature of 102.7° F. Most patients had no other symptoms noted, and none appeared toxic. Without specific antibiotic therapy, the fever lasted for a mean of 5 days. In six cases, paired antibody studies have been done, and a serologic response was seen in all.[9, 10] At Boston City Hospital, *M. hominis* is isolated from blood of 5% to 10% of women with fever after delivery.[8, 9]

It is also important to note the absence of *M. hominis* from the blood of normal puerperal patients. From four studies including approximately 200 women, *M. hominis* was isolated in the blood of only one patient who, interestingly, showed an antibody response.[8, 9]

U. urealyticum has been noted in the blood of fewer women with postpartum fever. Two cases from Israel were in women who had multiple symptoms, including pneumonia and diarrhea in one and sore throat and myalgia in the other.[12, 13] Eight additional cases were reported from one series (see below).[11] Serologic studies were not performed in any of these cases.

Recently, Lamey et al.[11] reported the largest series of symptomatic puerperal women with mycoplasmemia. Among 125 women with fever (>38° C) at any time after delivery, *M. hominis* was isolated in the bloodstream of nine (7.2%), *U. urealyticum* in eight (6.4%), and bacteria in 15 (12%). In five of these cases, bacteria and mycoplasmas were both isolated from the bloodstream. Blood cultures were also collected from 60 afebrile women 1 or 2 days post partum. None contained genital mycoplasmas. The difference in isolation rate of genital mycoplasmas in the blood between febrile and afebrile women was significant ($p < 0.005$). Of 16 women whose postpartum blood contained mycoplasmas, five (31%) had fever during labor, but three of these five women had simultaneous bacteremia. Fever during labor was significantly associated with organisms in the postpartum blood sample ($p < 0.005$).

Our study compared the rate of intrapartum mycoplasmemia in 81 women with clinical intra-amniotic infection and in 44 asymptomatic women. Although the groups were similar in several characteristics and both had prolonged rupture of the membranes, the intra-amniotic infection group had significantly longer rupture of the membranes, labor, and duration of internal fetal monitoring and a significantly higher cesarean section rate. Despite these differences, there was no significant difference in the rate of isolation of *U. urealyticum* in maternal blood. Isolation of *M hominis* in maternal blood was found only in the intra-amniotic infection group, but this occurred infrequently (2%) and was not significantly different from the rate in the asymptomatic group. Our overall rate of mycoplasma isolation in maternal blood in cases of intra-amniotic infection (16.0%) is similar to that observed by Lamey et al.[11] (13.6%) in maternal blood in cases of puerperal fever. Whereas those authors found *M. hominis* in 7.2% of blood cultures and *U. urealyticum* in 6.4%, we found *U. urealyticum* more commonly (13.6%) and *M. hominis* less commonly (2.5%). However, the major difference in observations is that Lamey et al. found no cases of mycoplasmemia among 60 asymptomatic puerperal women in contrast to our observation of frequent ureaplasmemia in asymptomatic women. This discrepancy may be explained by our asymptomatic group being in labor and the control group of Lamey et al. being postpartum. It is pertinent that McCormack et al.[8] found mycoplasmas in the blood transiently after delivery. Within 2 minutes, 15% had mycoplasmemia, but more than 10 minutes after delivery, only 2% had myco-

*A complete list of references will be sent to the reader on request.

Table IVB. Serologic and amniotic fluid culture results for *U. urealyticum* in 50 patients with intra-amniotic infection and 33 asymptomatic control subjects

	Group				
	Intra-amniotic infection, U. urealyticum*		*Asymptomatic,* U. urealyticum*		
Antibody response to U. urealyticum	*Positive (n = 22)*	*Negative (n = 28)*	*Positive (n = 14)*	*Negative (n = 19)*	*p*
Increase in optical density ≥ 0.2 units (n and %)	13 (59%)	15 (54%)	2 (14%)	4 (21%)	0.007
Acute optical density (mean ± SD)	0.408 ± 0.108	0.438 ± 0.121	0.484 ± 0.150	0.524 ± 0.163	NS
Change in optical density† (mean ± SD)	0.269 ± 0.123	0.260 ± 0.183	0.096 ± 0.091	0.111 ± 0.076	0.001

*Results of amniotic fluid cultures.

†Change equals convalescent minus acute.

plasmemia. Thus ureaplasma may be recovered transiently from the bloodstream of women in labor, at least in a group with prolonged membrane rupture.

When *M. hominis* was found in the blood, it was also found in the amniotic fluid. When *U. urealyticum* was found in the blood, it was isolated in the amniotic fluid in 16 of 19 cases (84%). Similarly, McCormack et al. found ureaplasma in the vagina in 77% of women whose blood contained this organism in the immediate puerperium.[8] These discrepancies may result from false negative amniotic fluid culture or from *U. urealyticum* having another portal of entry (for instance, the dilating cervix).

Neonatal blood cultures were subcultured for mycoplasmas as recommended by Caliando and McCormack,[5] but none was isolated. It is unclear whether this observation results from loss of organisms during culture or lack of invasiveness of genital mycoplasmas in the neonate. Mycoplasmas have been isolated from the blood of 7% of spontaneously aborted fetuses, but mycoplasmas were not isolated from neonatal blood, cerebrospinal fluid, or the interior of tissues at autopsy at Boston City Hospital.[14]

The second part of our study assessed serologic response to genital mycoplasmas in paired sera. An increase in specific antibody production is often taken as evidence of pathogenicity of an organism in a particular disease, yet, as with blood culture isolation, serologic techniques also have their limitations. A rise in antibody titer is usually indicative of recent exposure to antigen but is unable to indicate clinical disease or to localize the site of infection.

Serologic studies have not been previously performed in cases of clinical intra-amniotic infection (or clinical chorioamnionitis), but serologic investigations have been performed in patients with postpartum fever. In addition to the few serologic studies in reports of mycoplasmemia,[9, 10] the only other pertinent serologic data came from a recent survey at Boston City Hospital. Of 535 women who had vaginal deliveries, 23 had postpartum fever with no extragenital explanation.[15] A mycoplasmacidal antibody response to *M. hominis* was observed in 10 (44%). The strongest single predictor of these so-called "unexplained fevers" was an absent or low titer of antibody against *M. hominis*.

In the present study, we compared serologic response to *M. hominis* and to *U. urealyticum* in 50 women with intra-amniotic infection and in 33 asymptomatic control subjects. We used an enzyme-linked immunosorbent assay technique, which is rapid, reliable, and specific. As with blood culture data, the intra-amniotic infection patients in this part of the study had significantly longer labors, longer intervals from rupture of the membranes to delivery, and more cesarean deliveries. These potential confounding variables may have influenced the serologic results.

For *M. hominis*, the antibody response in patients with intra-amniotic infection and amniotic fluid positive for *M. hominis* is significantly different from patients with intra-amniotic infection and amniotic fluid negative for *M. hominis* and from control patients with negative amniotic fluid. *M. hominis* occurs infrequently in amniotic fluid of asymptomatic patients, although the response in these patients resembles that of the intra-amniotic infection group with *M. hominis* in amniotic fluid. It is not yet known whether there are responses to other organisms isolated in the amniotic fluid. It is also unclear why we detected a serologic response (mainly by "seroconversion") in some patients with amniotic fluid negative for *M. hominis*. Possibly these apparent seroconversions represent antigen exposure from the cervix. It is also possible that a small absolute difference in enzyme-linked immunosorbent assay reading may lead to a change, for example, from a negative to a weakly reactive value. These limitations of serologic techniques and of enzyme-linked immunosorbent assays are well recognized.

Although the intra-amniotic infection and control

groups were significantly different (labor, rupture of the membranes, cesarean section rate, etc.), we believe that these potential confounding variables have not influenced the serologic data for *M. hominis*. Indeed, we have demonstrated that route of delivery was not associated with serologic response and response was strongly associated with the presence of the organism and not simply with intra-amniotic infection.

For *U. urealyticum*, a different pattern was seen. Here the antibody response was significantly greater in the intra-amniotic infection group, but there was no difference between those with and without *U. urealyticum* in the amniotic fluid. This response is more difficult to interpret but may arise from (1) false negative amniotic fluid cultures for *U. urealyticum*, (2) antigen exposure from a site other than the amniotic fluid, or (3) lack of assay specificity. The first two possibilities are likely to make some contribution, as we saw negative amniotic fluid cultures for *U. urealyticum* in 15% (16 of 19) of women whose blood contained this organism. Manganese is present in Shepard's A,B agar, which was used for culture in the last part of the study. Robertson and Chen[16] have recently found that low concentrations (1 mmol/L) of manganese cation inhibited the growth of all standard strains of *U. urealyticum* to varying degrees. Perhaps use of manganese-containing agar contributed to false negative cultures of amniotic fluid. Assay specificity for *U. urealyticum* has been addressed previously.[6] Evidence supporting a high specificity includes excellent agreement with the metabolic inhibition assay (82% to 95% agreement in sera of patients with nongonococcal urethritis) and lack of cross-reactivity of *U. urealyticum* antigen with rabbit antisera of *M. hominis* or other mycoplasma species.

Our serologic data in the two women whose blood contained *M. hominis* is consistent with previous observations showing a nearly universal antibody response. In patients with ureaplasmemia, we saw an antibody response in one of two women with intra-amniotic infection and neither of two control women. The only other pertinent observation is that of McCormack et al., who observed no responses in any of seven women with transient ureaplasmemia after vaginal delivery. It was not noted whether any of these seven patients developed fever in the puerperium.

In sum, when *M. hominis* was found in the amniotic fluid or maternal blood, the patient was nearly always symptomatic. The high likelihood of serologic response in these patients lends further support to the pathogenicity of *M. hominis* in intra-amniotic infection. These observations parallel those in women with puerperal fever. When *U. urealyticum* is found in amniotic fluid or blood, patients are commonly asymptomatic. Although the pattern of serologic response is strongly associated with intra-amniotic infection, it is not associated with isolation of *U. urealyticum*. The observation that some patients with intra-amniotic infection have both ureaplasmemia and a serologic response supports the view that in these patients *U. urealyticum* is pathogenic. However, its pathogenicity in most cases of intra-amniotic infection remains unclear.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Richard H. Schwarz, Brooklyn, New York. Dr. Gibbs and his colleagues continue to pursue the role of genital mycoplasmas in obstetric infection. In their earlier study[1] they demonstrated that *M. hominis* could be isolated more frequently from amniotic fluid in patients with intra-amniotic infection than in that of control subjects. However, in that study as well as the current one there was a significant isolation of high-virulence bacteria in the intra-amniotic infection patients as well. Blood cultures in the current study were positive for *M. hominis* in only two patients, as compared to none of the control subjects, and although an interesting finding this is not statistically significant. The authors have attempted to further establish the significance of *M. hominis* by measuring serologic reactions. They have shown that serologic responses were more common in patients with *M. hominis* in amniotic fluid and intra-amniotic infection (70%) than in patients with intra-amniotic infection who did not have *M. hominis* in amniotic fluid (30%). Unfortunately, serologic responses also occurred in a significant percentage of those patients with positive amniotic fluid culture for *M. hominis* who did not manifest intra-amniotic infection (67%). All of this prompts a basic question: Has the study really established a pathogenic role for *M. hominis* in intra-amniotic infection? If so, is that role as a primary pathogen or does it act synergistically with the high-virulence bacteria so commonly present?

The data with regard to *U. urealyticum* are more difficult to interpret. Although recovery of this organism from amniotic fluid and blood was relatively common in asymptomatic women, seroconversion was significantly associated with intra-amniotic infection in the absence of *U. urealyticum* isolation. The authors have offered several explanations for this seeming disparity, concentrating for the most part on false negative amniotic fluid cultures.

Since the mycoplasmas are found so frequently in the vagina of asymptomatic women, it is not surprising that they have been implicated in a variety of reproductive problems from infertility to pelvic inflammatory disease to obstetric infections. Local recovery of the organism therefore does not establish a pathogenic role, and consequently further studies such as this are necessary to clarify that matter.

There are several questions I would like to pose for Dr. Gibbs.

1. Would it be possible to control for factors such as length of labor, duration of rupture of membranes, and intrauterine monitoring? The control subjects differed in all of these categories and it is possible that the rate of amniotic fluid recovery of mycoplasmas might be higher (even without intra-amniotic infection) after a longer exposure.

2. Serologic results were measured in three different ways. Would the results have differed if only strict seroconversion had been the criterion?

3. The authors suggest that mycoplasma seroconversion in patients without intra-amniotic infection may be the result of responses to cervical organisms. Are any data available on vaginal or cervical cultures in the study populaton?

4. The absence of *M. hominis* antibody has been shown to be predictive of postpartum fever. Is it also predictive of intra-amniotic infection?

5. In our own studies[2] of vaginal organisms in relation to other adverse pregnancy outcomes, namely, preterm delivery, mycoplasma was found to be associated with other significant organisms by analysis of covariance. Is a similar association possible in this study as well?

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Dr. Rudolph P. Galask, Iowa City, Iowa. I have three questions that I would like to ask.

Were the control women and the patients who subsequently developed endometritis evaluated?

The second question is when were the blood cultures obtained? Was this timed with fever in the subjects, and when were blood cultures obtained in the control women?

Were these done singly, in duplicate, or in triplicate?

The third question is how was the purification of the antigen done, and how did the authors determine specificity for the enzyme-linked immunosorbent assay technique described?

Dr. Kamran S. Moghissi, Detroit, Michigan. As Dr. Schwarz mentioned, the *U. urealyticum* has been incriminated in both infertility and repeated abortion.

My question is whether the previous reproductive histories of these women were known. Also can Dr. Gibbs speculate as to whether a positive serologic result might, in fact, have predated the current pregnancy?

Dr. Gibbs (Closing). I would like to thank Dr. Schwarz for his careful review of our work.

This certainly has been a complex topic considering the polymicrobial nature of this infection.

To his first question, that is, could we have controlled for those obstetric variables that were different between the symptomatic and asymptomatic groups, I would respond by saying that this would have been preferable, and, in fact, this was a technique that we used when we were analyzing the amniotic fluid microbiology, not for mycoplasmas only but for bacteria. We used patients who were matched with regard to those obstetric variables.

In these current studies there were significant differences in several obstetrical variables, and let me try to address how those differences might or might not have influenced the results.

Despite the differences, there were no significant differences in isolation of *U. urealyticum* from maternal blood. Isolation of *M. hominis* was found only in the blood of women with intra-amniotic infection, but the difference was not significant either. Thus I would not anticipate that controlling for those obstetric variables would have influenced the results.

In the serologic group of patients, there were again significant differences in those obstetric variables, but here I do not believe that those differences influenced the results. First of all, all sera were collected within a relatively short time of delivery, and the absolute difference, 6 or 12 hours, in those labor and delivery variables, was small compared with the half-life of IgG antibodies.

With regard to Dr. Schwarz' second question of strict seroconversion, we looked at that also. If we define seroconversion as a change from a negative value to a reactive value, then the distribution looks quite similar to the response of patients with a rise >0.2 optical density units.

The third question was do we have data on the vaginal and cervical cultures in this study population?

At the time we were performing this study, we were not doing concomitant vaginal and amniotic fluid cultures. We do know from a group of similar women in our obstetric population that ureaplasma and *M. hominis* occur more commonly, of course, in the lower genital tract than in the amniotic fluid, and currently we isolate *M. hominis* about 35% overall in the lower genital tract and ureaplasma about 60% overall.

The fourth question was whether the absence of antibodies to *M. hominis* is predictive of the development of intra-amniotic infection in view of observations by Platt et al. in Boston that absence of *M. hominis* antibodies was the most significant predictor of the development of postpartum fever.

We found no significant difference in the mean acute absorbance across the four groups.

I point out that Platt et al. used a mycoplasmachidal antibody titer and we used an enzyme-linked immunosorbent assay technique.

Although previous studies done by Cassell et al. have shown a good correlation between these two techniques, we did not find that a lower ELISA reading was predictive of intra-amniotic infection.

Dr. Schwarz' fifth question was: Is *M. hominis* associated with organisms that we have labeled high-virulence organisms in the amniotic fluid? In both this series and our original work, we found a high association between the presence of *M. hominis* and these high-virulence bacteria.

Here more than 75% of amniotic fluid samples with *M. hominis* also had 10^2 cfu/ml of high-virulence bacteria, and currently we are investigating the serologic response to these other common bacteria in amniotic fluid.

Dr. Galask asked whether we excluded patients who were asymptomatic in labor and who subsequently developed endometritis. Yes, they were excluded, and we also excluded patients who had transient postpartum fever.

One set of blood cultures for mycoplasma was collected during labor in the intra-amniotic infection group from patients who were febrile at the time. The sample was collected from control patients at the time that an amniotic fluid sample was obtained. This generally meant patients in labor with 10 to 12 hours of membrane rupture.

Dr. Galask's last question had to do with how the antigen was prepared, and how we tested for specificity since that is a question in particular with regard to the ureaplasma data.

The antigen was prepared from a whole cell lysate of *M. hominis* and *U. urealyticum* prepared with bicarbonate buffer and adherent to the plastic wells.

We looked very hard at the data, especially with regard to ureaplasma and questioned specificity. In previous work by Dr. Cassell the question of specificity was addressed in two ways.

First, there was a comparison between the mycoplasmacidal titer and the enzyme-linked immunosorbent assay response, and the agreement was good, generally between about 85% and 95%.

The other way that the assay specificity was compared was to look at the antigen preparation compared with known hyperimmune sera against other mycoplasma species. There again there was very little to suggest lack of assay specificity.

Dr. Moghissi asked whether we had information on the previous reproductive history of these patients. The majority of patients in both groups were young and nulliparous.

Could it be that the positive serologic result predated pregnancy? Yes, especially with regard to ureaplasma. We had a high level of reactivity in the serum in nearly all of our patients. This was a common antigen that our patients were exposed to.

With regard to *M. hominis* antigen, positivity was less common but still present in a considerable percentage of our patients.

The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo

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The ovulatory agent clomiphene citrate, a racemic mixture of the estrogenic zuclomiphene and antiestrogenic enclomiphene isomers, has been associated with gamete/embryo toxicity in various animal models. To assess the potential effects of these isomers on fertilization and early embryogenesis, 3200 embryos were obtained from mouse oocytes fertilized in vivo and in vitro and exposed to increasing concentrations of zuclomiphene, enclomiphene, and clomiphene citrate in culture. Fertilization rates ($p < 0.01$), blastocyst formation rates ($p < 0.02$), and degeneration rates ($p < 0.005$) were all adversely affected in a dose-dependent fashion. There were no statistically significant differences between the two isomers. Preincubation with 17β-estradiol did decrease the degenerative changes induced by clomiphene citrate but did not improve the decreased rate of blastocyst formation. These findings suggest adverse effects of a high concentration of clomiphene citrate and its isomers on fertilization and early mouse embryo growth that may have implications in the clinical use of this drug. (AM J OBSTET GYNECOL 1986;154:727-36.)

Key words: Clomiphene citrate, embryo toxicity, in vitro fertilization

Clomiphene citrate, a nonsteroidal triphenylethylene compound, is probably the most common agent used for induction of ovulation presently in medical use. Orally active, the drug is composed of two isomers, enclomiphene and zuclomiphene, formerly named cis-clomiphene and transclomiphene. Clomiphene citrate has a reported 60% to 80% ovulation rate and an associated 40% to 50% pregnancy rate, but there may also be an increased rate of spontaneous abortion associated with the use of clomiphene citrate.[1,2] The relative potencies of the two isomers in inducing ovulation in the human is unknown; however, in animal studies it appears that zuclomiphene is the more estrogenic of the two, although significant discrepancies do exist.[3-5] While the two marketed compounds (Clomid and Serophene) differ in their percent composition of the isomers, the clinical ovulation rate is reported to be unchanged.

Clomiphene citrate binds to the cytoplasmic estrogen receptor in numerous tissues including the hypothalamus, pituitary, and ovary.[5,6] It is thought to function by releasing the inhibitory effect of endogenous estrogens on gonadotropin secretion, possibly by alterations in gonadotropin-releasing hormone patterns. However, the precise mode of action in the hypothalamic-pituitary axis has yet to be determined. The evidence is clear, however, that clomiphene citrate may act as an estrogen or antiestrogen in the human, depending on the clinical situation when administered.[6] While the effects of the separate isomers zuclomiphene and enclomiphene have not been well studied in the human, most reports associate zuclomiphene with primarily estrogenic effects and enclomiphene with antiestrogenic activities.[3] It is interesting therefore that zuclomiphene citrate is considered to be the active isomer in clomiphene citrate when used for induction of ovulation.

At the ovarian level, clomiphene citrate has also been reported to have both estrogenic[7] and antiestrogenic[8] activity, and this again may correspond to the differential activity of the two isomers. Little is known of the effects of clomiphene citrate on the developing oocyte or on early embryogenesis. Early studies had suggested degenerative changes in oocytes and embryos in animals exposed to clomiphene citrate immediately after ovulation and that this damage was not reversed with estrogen administration.[9-11] More recently there have been contradictory findings of complete reversal of the effects of clomiphene citrate administered to mice before ovulation by concomitant administration of 17β-estradiol.[12] In another recent study using ovarian perfusion techniques, clomiphene citrate induced increased oocyte degeneration.[13] Decreased development of two-cell mouse embryos has also been reported from mouse oocytes pretreated with clomiphene citrate be-

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fore in vitro fertilization, but it is interesting that embryo growth from the two-cell embryo to the blastocyst stage was unaffected.[12] Unfortunately, the fertilization rate in the control groups was relatively low and the short pretreatment period may not accurately reflect the in vivo situation. No data are available on the effects of either zuclomiphene or enclomiphene citrate on the oocyte or embryo.

Considering the widespread clinical use of clomiphene citrate and the limited amount of data concerning the effects of the racemic mixture on either the fertilization process or early embryogenesis, it was of considerable interest to pursue the potential toxicity studies. The approach was to eliminate possible in vivo influences by exposing mouse oocytes fertilized either in vivo or in vitro to clomiphene, zuclomiphene, or enclomiphene citrate in an in vitro culture system, observing the rates of fertilization, embryo growth, and embryo degeneration. The findings suggest that there is a sensitivity to both clomiphene and its isomers from the time of fertilization until the two- to four-cell stage, which results in increased embryo degeneration and decreased cell growth. These alterations are sometimes not apparent until 24 to 48 hours after exposure and are not reversed with estrogen treatment.

Material and methods

Culture media. Hams F-10 with glutamine (Gibco Laboratories, Grand Island, New York), supplemented with calcium lactate, 0.8 mmol/L; sodium bicarbonate, 20 mmol/L; potassium bicarbonate, 5 mmol/L; penicillin, 100 IU/ml, and streptomycin, 100 IU/ml (Sigma Chemical Co., St. Louis, Missouri), was adjusted to an osmolarity of 278 ± 2 mosm/L and used as the primary culture medium. Heat-inactivated preovulatory human serum (56° C for 30 minutes) was added, 7.5% for the fertilization media and 15% for the growth media. The mixture was adjusted to pH 7.4 after equilibration with 5% carbon dioxide/5% oxygen/90% nitrogen and filter sterilized immediately before use. All cultures were carried out in welled Falcon culture dishes (Becton Dickinson Co., Oxnard, California) in a humidified 5% carbon dioxide/air incubator at 37° C.

Clomiphene, enclomiphene, and zuclomiphene citrates in powdered form were a gift from Merrell Dow Pharmaceuticals. A stock solution of clomiphene and each isomer was prepared by dissolving 3.0 mg of the citrate in 10 ml of culture media. All experimental oocytes or two-cell embryos were exposed for at least the first 24 hours of culture to clomiphene-, enclomiphene-, or zuclomiphene-supplemented media with subsequent transfer to drug-free growth media after 24 hours.

Animals. Female CB_6F_1 mice (Jackson Labs, Indianapolis, Indiana), 10 to 15 weeks of age, were used in all experiments. This hybrid is a cross between the Balb/c and C57B1/6 strains and consistently has provided oocytes with greater than 90% in vitro fertilization rates in our laboratory. Male mice were of the CD-1 strain (Jackson Labs), 8 to 10 months old, and had proved fertility. Ovulation induction was carried out by standard methods. On the first day of estrus determined by vaginal smear, the female mouse was administered 7.5 IU of pregnant mare serum gonadotropin (PMSG) intraperitoneally followed by 7.5 IU of human chorionic gonadotropin (hCG) intraperitoneally 72 hours later.

In vitro fertilization. The female mice were put to death by cervical dislocation 14 to 16 hours after the hCG injection. Oviducts were removed and the cumulus mass in each was dissected free. The oocytes were dispersed from the cumulus cells with 0.1% hyaluronidase. In most experiments oocytes from four mice were pooled and then randomly distributed into the control or experimental groups for subsequent fertilization.

The male mice were put to death by cervical dislocation and both epididymides were placed in unsupplemented culture media. Each epididymis was cut into six to eight pieces and incubated in 5% carbon dioxide/air at 37° C for 30 minutes. This cultured suspension was then filtered through gauze, the supernatant sperm suspension centrifuged at 150 × g for 7 minutes, and the pellet resuspended in 2 ml of culture media. Sperm motility and count were then evaluated and 0.5×10^6 motile sperm added to each dish containing eight to 10 oocytes. The ova and sperm were incubated for 24 hours in control or drug-supplemented fertilization media containing 0.1, 1, 10, 25, 50, and 100 μg/ml of clomiphene citrate or 0, 0.1, 0.5, 1, 10, 25, and 50 μg/ml of enclomiphene or zuclomiphene citrate. After 24 hours all fertilized oocytes were transferred into drug-free media. At 24-hour intervals the dishes were examined for fertilization rate, the number of blastomeres per embryo, blastocyst formation, and degenerative changes. At 96 hours, photomicrographs were taken for final evaluation. For each concentration of clomiphene, enclomiphene, and zuclomiphene citrate, 120 to 215 oocytes were tested.

In vivo fertilization. After the hCG injection, a male mouse was caged with each female mouse and left overnight. After mating was confirmed by observation of the vaginal plug, the female mice were put to death by cervical dislocation 24 to 26 hours after hCG administration. The oviducts were then excised and the fertilized ova were extracted and placed in growth media at 37° C. In most experiments, fertilized oocytes from four to six mice were pooled and then randomly distributed. A minimum of 100 oocytes were included in each group tested.

Clomiphene citrate–supplemented growth media containing 0, 0.1, 1, 10, 25, 50, and 100 μg/ml of the

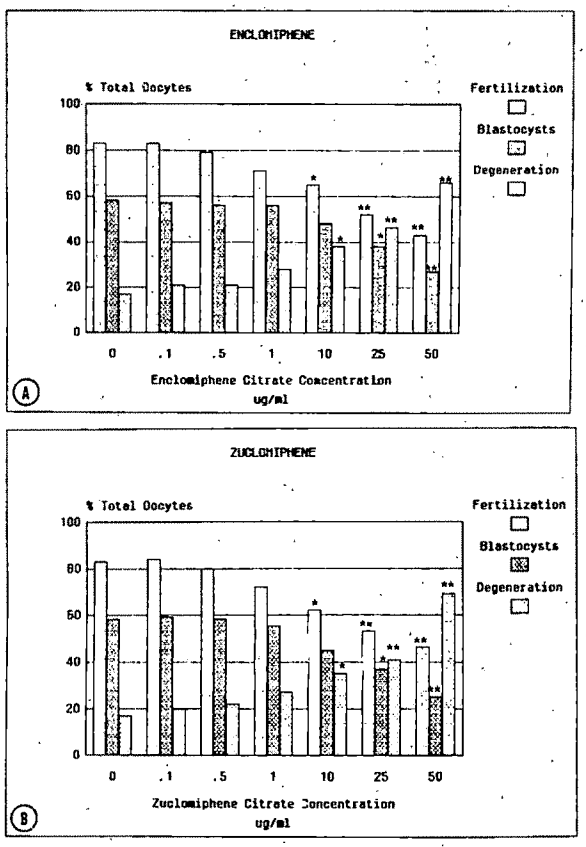


Fig. 1. *A*, The effects of enclomiphene citrate on the development of mouse oocytes fertilized in vitro. The oocytes were exposed to 0 to 50 μg/ml of enclomiphene citrate in culture media for the first 24 hours of the 96-hour incubation. Each group consists of 120 to 215 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.) *B*, The effects of zuclomiphene citrate on the development of mouse oocytes fertilized in vitro. Conditions are identical to those in *A* except for the drug. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.)

drug and enclomiphene and zuclomiphene citrate–supplemented media containing 0, 0.1, 0.5, 1, 10, 25, or 50 μ/ml of the drug were used as the incubation media for 24 hours followed by transfer to unsupplemented growth media. At 24-hour intervals the embryos were examined for blastocyst formation; the number and morphology of the blastomeres were also noted. At 72 hours, photomicrographs were taken of the embryos for final evaluation. In studies evaluating exposure to clomiphene citrate for 72 hours, the embryos were transferred every 24 hours into media containing 0.1, 1, and 10 μg/ml of clomiphene.

Statistical analysis was performed with the test of proportions with z score.

Results

In vitro fertilization. Mouse oocytes were exposed to increasing concentrations of zuclomiphene citrate, enclomiphene citrate, and clomiphene citrate, ranging from 0 to 100 μg/ml during the fertilization process.

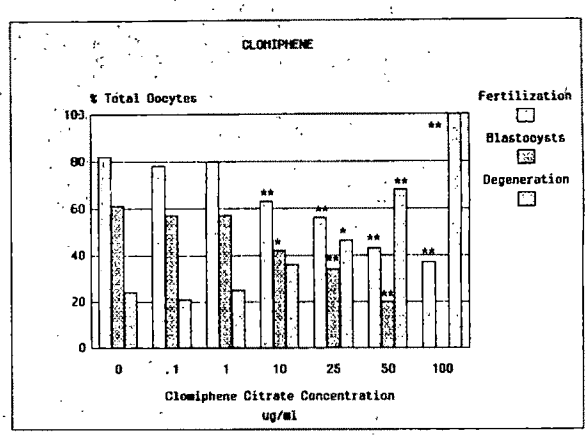


Fig. 2. Effects of clomiphene citrate on the development of mouse oocytes fertilized in vitro. The oocytes were exposed in culture to 0 to 100 μg/ml of clomiphene citrate for the first 24 hours of the 96-hour incubation. Each group consists of 100 to 250 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.)

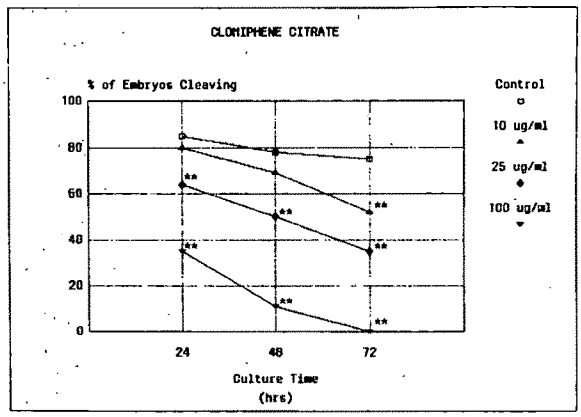


Fig. 3. The rate of progressive mouse embryo cleavage after in vitro fertilization and clomiphene citrate exposure. The oocytes have been exposed in culture to 0 to 100 μg/ml of clomiphene citrate for the first 24 hours. Each group consists of 100 to 250 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.)

The fertilization rate of the untreated oocytes, based on achieving a two-cell embryo, was 83%. Fig. 1 summarizes the results comparing the rates of oocyte fertilization, embryo growth as assessed by blastocyst formation, and degeneration where exposed to enclomiphene (Fig. 1, *A*) and zuclomiphene (Fig. 1, *B*) citrates in the culture media. For comparison, Fig. 2 summarizes the same situation with a racemic mixture of clomiphene citrate.

As noted with both the racemic mixture and the isomers, the fertilization rate and the embryo growth rate both declined in a dose-dependent fashion as the drug concentration increased. These changes reached statistical significance ($p < 0.05$) at a concentration of 10 μg/ml. There was no statistically significant difference between the two isomers in either fertilization rates or

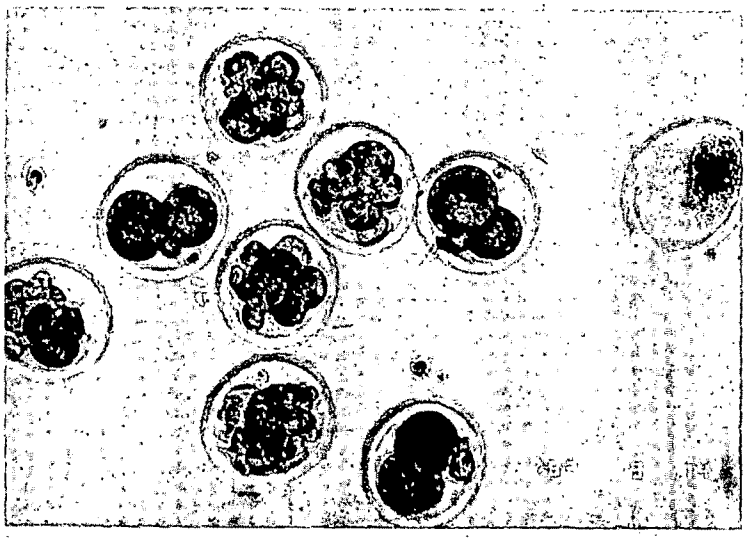


Fig. 4. The effect of enclomiphene citrate exposure on cultured mouse embryos. These embryos fertilized in vitro were exposed to 25 μg/ml of enclomiphene citrate 43 hours previously and are representative of degenerated cells. (Original magnification × 300.)

Table I. Degeneration of 4- to 8-cell mouse embryos with exposure to enclomiphene and zuclomiphene citrate

Concentration (μg/ml)	*No. of embryos*	*Degenerated*	
		n	*%*
Enclomiphene			
0	150	5	3.3
1	105	5	4.8
10	90	13	14.4*
25	46	10	21.7†
50	24	11	45.8†
Zuclomiphene			
0	150	5	3.3
1	98	4	4.1
10	95	13	13.7*
25	51	12	23.5†
50	27	13	48.1†

*$p < 0.05$ versus control value.
†$p < 0.001$ versus control value.

blastocyst formation rates. As seen in Fig. 3, the rate of progressive embryo cleavage was detectably slower as early as 24 hours with clomiphene citrate concentrations ≥25 μg/ml and became more pronounced after each additional 24 hours. The actual percent of two-cell embryos reaching the blastocyst stage decreased with the increasing concentrations of either the racemic clomiphene citrate or its isomers from 68% to 72% in nontreated culture to 0% at 100 μg/ml concentrations.

The embryo degeneration rate also demonstrated a dose-dependent ratio but increased with increasing drug concentrations. This occurred in both the racemic clomiphene citrate and the isomers enclomiphene citrate and zuclomiphene citrate. Embryos were not classified as degenerated unless the structural alternatives were visible at low microscope power and consisted of primarily pyknotic nuclei that had retracted from the zona pellucida and multiple blebs from the blastomeres. Arrested growth of the embryo was not classified as degeneration until the cellular changes were visualized. An example of degenerated embryos exposed to 25 μg/ml of enclomiphene are seen in Fig. 4.

Table II. Degeneration of 4- to 8-cell mouse embryos with exposure to clomiphene citrate

Clomiphene concentration (μg/ml)	*No. of embryos*	*Degenerated*	
		n	*%*
0	132	6	4.5
1	89	3	3.4
10	80	13	16.2*
25	65	16	24.6†
100	15	15	100†

*$p < 0.05$ versus control value.
†$p < 0.001$ versus control value.

Degeneration of embryos reaching the four-cell stages was more frequent in embryos that had been exposed to either zuclomiphene or enclomiphene citrate than in the nontreated group, reaching significance at concentrations >10 μg/ml (Table I). There was no statistical difference between the two isomers in their effects on delayed embryo degeneration. In separate studies, clomiphene citrate demonstrated similar findings, as seen in Table II.

In vivo fertilization. To minimize the potential effect of clomiphene or the isomers on the fertilization process as the primary embryo-toxic effect, two-cell embryos were obtained from mice after natural mating

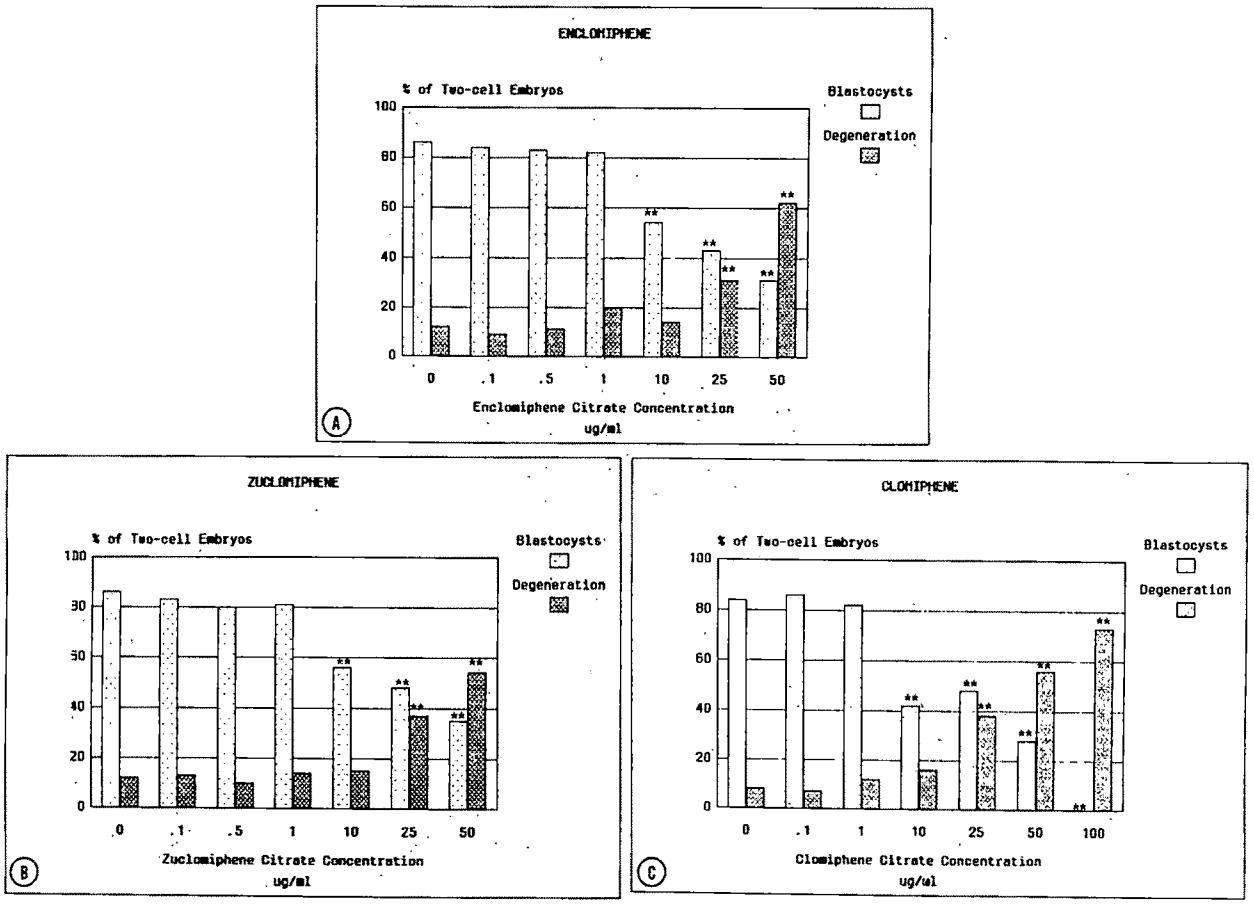


Fig. 5. *A*, The effects of enclomiphene citrate on the development of mouse oocytes fertilized in vivo. The fertilized oocytes underwent a 24-hour exposure to 0 to 50 μg/ml of enclomiphene citrate in the culture media. Each group consists of 100 to 180 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.) *B*, The effects of zuclomiphene citrate on the development of mouse oocytes fertilized in vivo. The fertilized oocytes underwent a 24-hour exposure to 0 to 50 μg/ml of zuclomiphene citrate in the culture media. Each group consists of 100 to 180 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.) *C*, The effects of clomiphene citrate on the development of mouse oocytes fertilized in vivo. The fertilized oocytes underwent a 24-hour exposure to 0 to 100 μg/ml of clomiphene citrate in culture media. Each group consists of 100 to 150 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.)

and exposed to zuclomiphene, enclomiphene, and clomiphene citrates for the first 24 hours of culture. Blastocyst formation was determined at 72 hours; in the untreated group 86% of the two-cell mouse embryos reached the blastocyst stage during that time. Degeneration of the embryos in the untreated groups during the same time period was 12%.

Embryos resulting from oocytes fertilized in vivo and exposed to enclomiphene (Fig. 5, *A*), zuclomiphene (Fig. 5, *B*), or clomiphene citrate (Fig. 5, *C*) in culture media demonstrated findings similar to those of the oocytes fertilized in vitro. There was a significant decrease in the blastocyst formation beginning at a drug concentration of 10 μg/ml ($p < 0.01$) in all three drug groups. Again, there was no significant difference between the two isomers in the decreased blastocyst formation rate. There were no blastocysts formed in the clomiphene citrate–treated group at a concentration of 100 μg/ml ($p < 0.001$). Growth rates, the percent of embryos that have divided at 24-hour intervals, is shown in Fig. 6. Enclomiphene and zuclomiphene citrate appear to alter growth rates beginning at 10 μg/ml at 48 hours.

An interesting observation is the lack of significant change in the number of degenerating embryos at the 10 μg/ml concentration of either enclomiphene or zuclomiphene. This results from increased numbers of drug-exposed embryos undergoing maturation arrest but not degenerating.

To determine whether additional exposure (>24

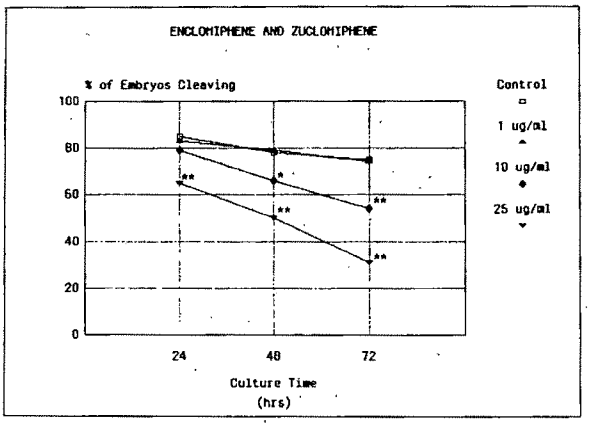


Fig. 6. The rate of progressive mouse embryo cleavage after in vivo fertilization and enclomiphene and zuclomiphene citrate exposure. The fertilized oocytes were exposed to 0 to 50 μg/ml of either enclomiphene or zuclomiphene citrate for the first 24 hours of culture. Each group represents pooled results of the individual isomers and consists of 180 to 260 oocytes. (* = $p < 0.01$ versus control value; ** = $p < 0.001$ versus control value.)

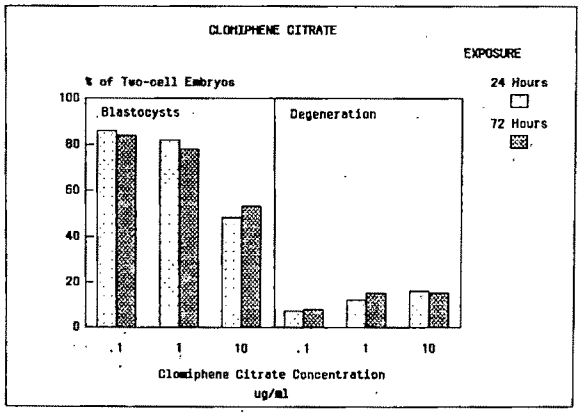


Fig. 7. The effect of length of clomiphene citrate exposure on the development of mouse oocytes fertilized in vivo. The fertilized oocytes were exposed to 0.1, 1, and 10 μg/ml of clomiphene citrate for 24 and 72 hours. Each group consists of 100 to 150 oocytes. There were no significant differences between the groups.

hours) of the cultured embryos to clomiphene citrate had any additional effect, two-cell embryos were exposed to 0.1, 1, and 10 μg/ml of clomiphene citrate for the entire 72-hour culture period (Fig. 7). There does not appear to be any additional effect in terms of blastocyst formation or embryo degeneration measured at 72 hours, whether the embryos are exposed for 24 or 72 hours.

To assess whether exposure of the mouse two-cell embryo to 17β-estradiol would ameliorate or prevent the embryo-toxic effects of clomiphene citrate, 17β-estradiol was added to the culture media at a concentration of 1 and 10 ng/ml for the first 24 hours. As seen in Fig. 8, the addition of the 17β-estradiol had no adverse effect when these embryos were compared with

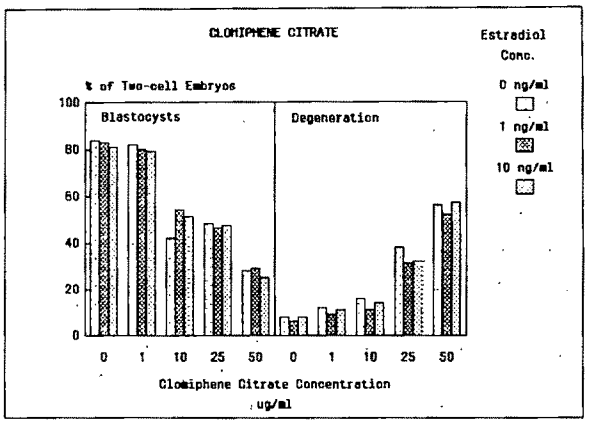


Fig. 8. The effect of concomitant 17β-estradiol treatment to mouse embryos fertilized in vivo and exposed to clomiphene citrate. The fertilized oocytes were exposed to 0 to 50 μg/ml of clomiphene citrate and 0, 1, or 10 ng/m of 17β-estradiol for the first 24 hours of culture, and blastocyst formation and degeneration were assessed at 72 hours.

untreated ones in terms of either blastocyst formation or embryo degeneration, and at clomiphene citrate concentrations of 1, 10, and 25 μg/ml, while there was no significant improvement in blastocyst formation, there was a generalized decrease in degeneration rates where 17β-estradiol was included in the culture media. This trend was not statistically significant. At concentrations >25 μg/ml of clomiphene citrate, concomitant treatment with 17β-estradiol had no effect.

Comment

Clomiphene citrate is a synthetic, nonsteroidal compound widely used as an ovulatory agent in clinical practice and has seen increasing use in in vitro fertilization–embryo transfer programs as a superovulatory agent. A discrepancy between ovulation and pregnancy rates and an increased spontaneous abortion rate during clomiphene citrate therapy has prompted concern over its potential embryo-toxic effects. Clomiphene citrate has been shown to bind to cytoplasmic estrogen receptors in multiple tissues[5,6] and may act as either an estrogen agonist or an antagonist, depending on the site, the estrogen level, and the length of exposure.[6] Since clomiphene is actually a mixture of the isomers zuclomiphene and enclomiphene, which are usually reported to display estrogenic and antiestrogenic activities, respectively,[3] it is of interest to determine whether the isomers display different effects on fertilization and embryo growth when compared with the racemic clomiphene citrate mixture.

The effects of clomiphene citrate at the level of the gamete or early embryo are limited. Early studies suggested degenerative changes in oocytes and embryos from animals exposed to clomiphene citrate after ovulation.[9-11] These effects did not appear to be reversed with estradiol treatment. In a more recent study

with PMSG-hCG–injected mice,[12] preovulatory subcutaneous injection of clomiphene citrate resulted in decreased mating activity and ovulation and delayed embryo development, all of which were reversed with 17β-estradiol. The authors also found a dose-dependent decrease in blastocyst formation and an increase in degenerated and unfertilized ova in mouse oocytes pretreated for 6 hours with clomiphene citrate before in vitro fertilization. They also noted that the growth rate from the two-cell embryo to the blastocyst appeared to be unaffected by the short pretreatment with clomiphene citrate. No data are available on the effects of zuclomiphene or enclomiphene on the ovary, gametes, or early embryos.

Studies that use in vivo exposure to clomiphene citrate have several potential limitations. Clomiphene does not induce ovulation in most animal species,[3] and depending on the tissue and species, both clomiphene and the individual isomers may act as either estrogens or antiestrogens or both.[3-6, 13] This complicates interpretation of results from in vivo models since additional treatment is needed to induce ovulation, most studies resorting to PMSG-hCG regimens. A second major problem is the lack of information on drug distribution in the human, including complete lack of data on genital tract tissue levels (for example, follicular fluid or ovarian, tubal, or uterine tissue). Half-life estimates (~5 days) from labeled tracer studies[14] have suggested that the clomiphene citrate should be present well after midcycle in the human, with 2- to 3-hour postdrug plasma levels in the range of 10^{-7} mol/L.[15] The possibility of accumulation or sequestration in tissues with high estrogen receptor levels also cannot be excluded.

Little is known about the fate of zuclomiphene and enclomiphene after oral administration. In a survey of five patients, plasma concentrations of each isomer were 9 to 80 ng/ml approximately 3 hours after a single 100 mg oral dose.[15] A preliminary report also suggests a differential elimination rate of the two clomiphene isomers such that plasma concentration of the zuclomiphene citrate was essentially identical for the 96-hour study period whether the oral dose contained 40% zuclomiphene (Clomid) or 55% zuclomiphene (Serophene).[16] Zuclomiphene citrate was also easily quantitated in plasma 28 days after a single 50 mg oral tablet. Since it appears that in the human, tissues have a prolonged exposure to clomiphene citrate and the currently used animal models do not respond by ovulation when treated with clomiphene citrate, we were interested in determining whether a more prolonged and extracorporeal exposure to clomiphene citrate and individually its isomers zuclomiphene and enclomiphene citrates would provide a clearer picture of any embryotoxic effects of the drug.

The results reported here suggest a significant effect of oocyte or embryo exposure to clomiphene citrate and its isomers as determined by fertilization rates, blastocyst formation, and degeneration rates. These effects are first apparent at concentrations of 5 to 10 μg/ml of either isomer in the culture media, which is close to the estimated plasma range. There does not appear to be a significant difference between either of the isomers or the racemic mixture in terms of the magnitude of the adverse effects. This would also suggest that the effects are not directly related to the estrogenic or antiestrogenic properties of the compounds. Supporting this conclusion are the results of the experiments demonstrating no protective effect of 17β-estradiol on the clomiphene citrate–induced decreases in embryo growth rates.

A second and important finding was the delayed effect of enclomiphene, zuclomiphene, or clomiphene citrate on embryo growth. As shown in Figs. 3 and 6, at lower concentrations there was a 24- to 48-hour interval between drug exposure and a decline in growth rate and an increase in degeneration rate for oocytes fertilized both in vitro and in vivo. This transition time is evidenced by the decreased rate of blastocyst formation at 10 μg/ml of enclomiphene or zuclomiphene but essentially no significant change in the degeneration rate. Growth has been significantly slowed and, with follow-up for longer periods of time, degenerative changes would undoubtedly appear. This obviously may also pertain to lower dosages of either clomiphene citrate or its isomers in that drug-induced alterations at drug concentrations of 0.1 or 1 μg/ml may have their effect at or beyond the blastocyst stage. These results differ critically from those reported by Laufer et al.,[12] who suggested that there are no changes in growth rate after the embryo reaches the two-cell stage. However, the oocytes used in that study were pretreated with clomiphene citrate for only 6 hours so that the oocyte was exposed for a short time to the drug. Second, the rate of fertilization was relatively low (<60%) in that study, making interpretation more difficult.

Exposure of the embryos to either clomiphene citrate or its isomers for 72 hours instead of 24 hours did not alter either the rate of blastocyst formation or the embryo degeneration rate for the 72- to 96-hour time period studied. Whether postblastocyst development would be affected is speculative at this time, but obviously embryo transfer of these exposed embryos to recipient pseudopregnant mice would help address that issue. It therefore appears that at least with early embryo-toxic events, the mouse embryo is sensitive to clomiphene citrate or its isomers through the two- to four-cell stage. The alterations in growth rate and degeneration also are not dependent on changes at fertilization, since exposure of the embryos fertilized in vivo to clomiphene citrate or its isomers yields results similar to those of the group fertilized in vitro. Late exposure of mouse embryos (≥eight cells) does not ap-

pear to slow embryo growth rate or increase degeneration rates until concentrations of 25 μg/ml or more of zuclomiphene or enclomiphene are used.

The present study clearly demonstrates a dose-dependent decrease in fertilization and embryo growth and an increase in embryo degeneration when either oocytes fertilized in vitro or two-cell embryos fertilized in vivo are exposed in vitro to zuclomiphene, enclomiphene, or the racemic mixture clomiphene citrate. These findings would correspond to those of earlier work with in vivo models. On the other hand, the evidence presented here clearly suggests that exposure to zuclomiphene, enclomiphene or, clomiphene citrate either during fertilization or through the two- to four-cell stage has potential long-term embryo-toxic effects, implying that even embryos demonstrating early progressive cleavage may later undergo diminished embryo development and have an increased degeneration rate. Subtle changes within the embryo may eventually lead to the embryo's death long after initial exposure to the drug.

The clinical implication of these data is that the embryo-toxic effects of clomiphene citrate may in part be responsible for the discrepancy between ovulation and pregnancy rates and the increased spontaneous abortion rate. While clomiphene citrate has enjoyed widespread use as an ovulatory agent, care must be taken to use the lowest effective dose to minimize exposure of the gametes and early embryos to the drug.

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Discussion

Dr. Daniel R. Mishell, Jr., Los Angeles, California. Clomiphene citrate is widely used in in vitro fertilization programs to induce maturation of more than one follicle. In these programs it has been shown that the probability of effecting a successful pregnancy is directly correlated with the number of embryos transferred. Because earlier reports summarizing the experience of the use of clomiphene citrate to induce ovulation in anovulatory women have shown a discrepancy between presumptive ovulation rates and pregnancy rates, it has been postulated that clomiphene citrate may have a toxic effect on the developing embryo. Such an effect could also account for the low pregnancy rate after human embryo transfer. This study was undertaken in an attempt to determine if this agent did indeed have an adverse effect on fertilization and embryo growth using the mouse model. After completion of this study, it was concluded that both clomiphene citrate and each of its two isomers had an adverse effect on fertilization, blastocyst formation, and embryo development of the mouse because each of these parameters was altered adversely in a dose-dependent manner as the concentration of each of these three agents increased. Nevertheless, these data should not be used to indicate that these alterations in parameters of in vitro mouse fertilization and embryo growth rates are also responsible for the differences between ovulation and pregnancy rates reported in women treated with this drug to induce ovulation. The authors also state that there is an increased spontaneous abortion rate in human pregnancy after the use of clomiphene citrate, and the embryotoxic effect observed in this study may be the responsible factor. The data generated do not support such a conclusion for the following reasons.

First, adverse effects on fertilization, embryo development, and blastocyst formation were observed only with drug concentrations of ≥10 μg/ml when clomiphene or its isomers were added directly to the culture media in this in vitro system. Three hours after ingestion in the human, serum concentrations of clomiphene

citrate have been found to be in the 9 to 80 ng/ml range and thus the concentrations to which human gametes and embryos are exposed several days after ingestion of the last tablet are at most <1% of the minimally significant toxic dose observed in this mouse study.

Second, since no positive controls were used, it has not been proved that the adverse effects observed were due to the drug itself. They may have been due to alterations in metabolic parameters, such as osmolarity and pH, in the tissue culture system produced by this relatively high concentration of drug. Maintenance of constancy of these and other environmental parameters is extremely important to ensure optimal rates of fertilization and embryo development in any in vitro system. Dr. Kim, were these and other parameters measured in this study and, if so, did they remain constant after addition of different concentrations of the drug?

Third, it is important to know whether a sufficient number of repetitive trials with a sufficient number of embryos were performed to provide statistical validity. Variation in numbers of animals and gametes used as well as culture conditions can influence the outcome of in vitro fertilization. How many repetitive experiments were performed in each study group and what was the variability of results among the experiments?

Finally, the references used by the authors to demonstrate that ovulation induction with clomiphene citrate in the human is associated with a lower pregnancy rate than fertilization rate and a higher rate of spontaneous abortion were reports from the 1960s in which strict criteria for enrollment of subjects, dosage of drug, and duration of therapy were not used. The results are different in more recent studies in which enrollment criteria, dosage, and follow-up were better controlled and actuarial statistical techniques used. In a 10-year follow-up study of 428 women treated with an individualized variable treatment regimen, Gysler et al.[1] found that although the overall incidence of ovulation was 85.3% and that of pregnancy was 42.8%, the pregnancy rate was 88.2% among couples with no other infertility factor who ovulated with clomiphene citrate treatment.

Hammond et al.[2] recently reported similar results. During a 5-year study of 159 patients treated with clomiphene citrate, 86% ovulated and 49% conceived, but with the use of life-table analysis they found that the pregnancy rate over time after clomiphene citrate treatment, provided no other infertility factors were present, was nearly identical to that of normal fertile couples. In their study, if the women did not discontinue treatment, the cumulative conception rate after 10 cycles of clomiphene citrate treatment was more than 95%, similar to the rate of conception in this same time period among normal women discontinuing contraception to conceive. In these two studies the spontaneous abortion rate was 14% and 15%, respectively, similar to that observed in other studies of large numbers of previously infertile patients who conceived and similar to the incidence of clinically recognized spontaneous abortion in all human populations.

Thus these two reports do not indicate that the use of clomiphene citrate administered orally before ovulation has an adverse effect on fertilization or embryo development in the human.

The clinical implications of data obtained from animal studies using an in vitro system should not be overinterpreted. In disagreement with the conclusion of the essayist, the results of the study reported today showing adverse effects on fertilization and embryo development of high dosages of clomiphene citrate when added directly to a mouse in vitro system should not be extended to interpret effects of the clinical use of this highly effective ovulation-inducing agent.

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DR. ROBERT B. JAFFE, San Francisco, California. In addition to the relatively high doses of clomiphene that were used in this study, the time of exposure in the in vivo experiments was far different from the time that we use clomiphene in inducing ovulation in women, and I wondered why that particular timing of administration was employed.

DR. GERE S. DIZEREGA, Los Angeles, California. A number of years ago a couple of reports demonstrated morphologic abnormalities in karyotypes of neonates from patients who had received clomiphene citrate for ovulation induction. Although these pregnancies resulted in clinically normal infants, some of the karyotypes contained morphologic abnormalities that were classified as "gene breakages." I wondered many times since then, Dr. Kim, if someone had a chance to perform a dose-response study as you did with clomiphene in sufficient doses to induce gross abnormalities that would be an opportunity to readdress those bothersome data.

Did you have a chance to do karyotyping of any of the embryos, and if so, were there any abnormalities?

DR. KIM (Closing). I agree with Dr. Mishell that any in vitro data should be applied to the in vivo situation with many considerations.

As to the question concerning the condition of the culture, our preliminary study had shown no change in the culture media when clomiphene citrate was added. Therefore we did not check the osmolarities or other elements in each experiment. However, pH was measured every day, and there was no gross change in the pH.

In terms of variability of the experiments, we performed one series of experiments including the control and study groups in 1 day to eliminate the variability of the experimental conditions. The results were pooled for the statistical analysis. The critical question

was raised concerning the concentrations of the drug used in the study, whether it is a physiologically acceptable range or not. Unfortunately, there are no data available regarding follicular or plasma concentration of clomiphene citrate in humans or animals since assay technique was such that one could not measure accurately. However, there have been some metabolic studies of clomiphene citrate from which we can learn.

Harmon and Blackman (see reference 15) published the high–performance liquid chromatography method of measuring clomiphene citrate, which is what Dr. Mishell quoted. In that study a single dose of 100 mg administered orally would give approximately a 30 to 80 ng/ml concentration, and concentrations of the isomers are variable because metabolic clearance rates of these two isomers are quite different. Mikkelson et al. (see reference 16) also showed that after a single dose of 50 mg of clomiphene citrate was given to a healthy woman, zuclomiphene, for example, was detectable in plasma as long as 14 days later. Other investigators have demonstrated that 50% of carbon 14–labeled clomiphene citrate was excreted in 5 days. Considering the half-life of 5 days and the concentration from a single 100 mg dose and pharmacologic cumulative effects of the drugs, when more than a 150 mg daily dosage is given for 5 days, plasma concentration will be somewhere between 1 and 10 μg/ml. This was only a theoretical estimation. For that reason we decided to study the range of 0.1, 10, and 25 μg/ml.

The question raised by Dr. Jaffe regarding a high dosage of clomiphene is important. Certainly 25 μg/ml of clomiphene citrate is not a physiologic but a pharmacologic level. The idea of the project was to show if there was a dose-dependent factor in terms of its effect on the embryos. Therefore a high dose range was tested.

Finally, Dr. diZerega brought up an important question regarding genetic aspects of the embryos exposed to clomiphene citrate. We have not done such a study. Karyotyping would be the important next step to investigate the cytotoxicity of this drug.

Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes

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Four experiments evaluated the sensitivity and specificity of molecular techniques to detect human Y chromosome deoxyribonucleic acid. In experiment I, electrophoretic separation of normal male deoxyribonucleic acid fragments after digestion with endonuclease *Hae* III revealed two male-specific bands of 3.4 and 2.1 kilobase (kb). These bands were not visible if the fraction of male deoxyribonucleic acid in mixed samples was less than 0.3. In experiment II, by means of a repetitive copy Y deoxyribonucleic acid probe (pS4) mapped to Yq12, a male-specific 2.3 kb band was detectable in mixtures of 2.5 ng of male deoxyribonucleic acid and 997.5 ng of 45,X female deoxyribonucleic acid. In experiment III, hybridization with the pS4 probe was performed on the deoxyribonucleic acid of 20 subjects with a normal or a variant Y chromosome. In experiment IV, deoxyribonucleic acid from the same subjects was hybridized to a single copy probe (4B-2) mapped to the Yq11 region. Deoxyribonucleic acid from category A subjects (n = 8) with cytologically normal Y chromosomes hybridized to both deoxyribonucleic acid probes. Deoxyribonucleic acid from category B subjects (n = 2), including a variant Y chromosome that was negative for Q-banding but positive for C-bands, hybridized with the distal pS4 and proximal 4B-2 probes. Deoxyribonucleic acid from category C subjects (n = 10) with variant Y chromosomes uniformly negative for Q- and C-bands, did not hybridize with the pS4 probe. Deoxyribonucleic acid from three of the 10 category C subjects did hybridize to the more proximal sequence–detecting 4B-2 probe. Deoxyribonucleic acid from the remaining seven subjects in category C did not hybridize with either of the deoxyribonucleic acid probes. (Am J Obstet Gynecol 1986;154:737-48.)

Key words: Normal/variant Y chromosomes, deoxyribonucleic acid (DNA) probes, pS4, 4B-2

Detection of a minority population of cells with a Y chromosome in a putative mosaic 45,X individual, or clarification of a chromosomal fragment suspected to be Y, by cytogenetic techniques is difficult and frequently uninformative. The clinician can sometimes resolve the dilemma only by prophylactic extirpation of the dysgenetic gonads in individuals at risk for having an undetected Y chromosome in a small fraction of cells. Cytogenetic techniques to identify and study the Y chromosome can now be complemented by the use of Y-specific deoxyribonucleic acid (DNA) probes.

Cooke and associates were the first to recognize that DNA from males which is digested with the restriction enzyme *Hae* III yields two male-specific fragments with molecular sizes of 2.24×10^6 (3.4 kilobase pairs) and 1.6×10^6 (2.1 kb pairs), respectively. These two fragments are composed of moderately repetitive DNA sequences that are visible on agarose gels stained with ethidium bromide and viewed under short-wave ultraviolet light (250 nm).[1] The 3.4 kb (DYZ1*) fragment class has been mapped to Yq11-q12. This region includes the heterochromatic and euchromatic portion of the long arm of the human Y chromosome. The 2.1 kb (DYZ2*) fragment class has been mapped exclusively to the heterochromatic region of the Y chromosome Yq12 → qter.[2,3] These reiterated male-specific sequences (3.4 kb, 2.1 kb) constitute approximately 40% and 20%, respectively, of the DNA of the human Y chromosome.[4,5]

Recently Disteche et al.[6] constructed a Y-specific DNA probe from a 850 base pair (bp) nucleotide sequence derived from the 2.1 kb *Hae* III repetitive DNA fragment visible on ethidium bromide–stained gels. After digestion of DNA with *Mbo* I, hybridizing Southern

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Table I. Tabulated clinical, cytogenetic, and DNA data on 20 study subjects*

Case No.	*Age at time of study*	*Phenotype*	*Karyotype (% 46,XY cells)*	*Total cells counted (n)*	*Morphologic structure of Y*
Category A					
1	40	Female with RSG†	10	50	▫
2	25	Female with RSG	16	50	▫
3	20	Female with RSG	20	50	▪
4	15	Female with RSG	88	50	▪
5	21	Female with RSG	82	50	▫
6	3	Female with unilateral labial testis	84	50	▭
7	3	Male with bilateral scrotal testes	87	100	r
8	25	Female with unilateral abd testis	94	50	r
Category B					
9	3 mo	Male with bilateral testes	100	50	del(Y)(q12)
10	15	Female with unilateral abd testis	94	50	dic(Y)(qter→p11::p11→qter)
Category C_1					
11	25	Female with RSG	14	50	del(Y)(q12)
12	7	Female with RSG	64	50	del(Y)(q12)
13	8 mo	Female with RSG	40	50	del(Y?) acentric fragment?
Category C_2					
14	30	Female with RSG	52	50	r(Y?)(p11q12)
15	7	Female with unilateral labial testis	50	50	del(Y)(q12)
16	28	Female with RSG	18	50	del(Y?) acentric fragment?
17	40	Female with RSG†	68	50	del(Y?) acentric fragment?
18	15	Female with RSG	92	50	del(Y)(q12)
19	15	Female with bilateral† testes and unilateral streak	76	50	del(Y)(q12) var(22)(p13,QFQ 4,5)
20	30	Male azoospermia with bilateral testes	72	50	del(Y)(q12)

RSG, Rudimentary streak gonads.

*Category A subjects (1 through 8) had cytologically normal Y chromosomes and hybridized with both pS4 and 4B-2 probes. Category B (subjects 9 and 10) consisted of one subject with loss of Q-banding but intact C-bands and one subject with a dicentric Y chromosome in multiple cell lines. Subject 9, positive for C-heterochromatin, still hybridized with both probes. Category C subjects with del(Y)(q12) fell into two categories depending on whether they hybridized with the proximal 4B-2 probe. Subjects in the C_1 category (subjects 11-13) hybridized with the 4B-2 sequences, whereas the remaining subjects (14-20) did not demonstrate a 4B-2 band.

†Dysgenetic tumors: dysgerminoma (No. 1), gonadoblastoma (No. 17), and embryonal carcinoma (No. 19).

blots to this repetitive Y probe (pS4) allows one to discern a clear distinction between male and female DNA, with male DNA exhibiting a sex-specific 2.3 kb band of hybridization. Recent evidence suggests that the upper limits of the pS4 sequences on the Y long arm are defined by the upper limits of C-banding and hence are contained within Yq12.[7]

Burke et al.[8] isolated a single copy DNA probe, 4B-2, which maps to the proximal portion of the long arm of the human Y chromosome (subcentromeric → q11), placing it entirely in the euchromatic region.

DNA-based techniques to identify Y chromosome DNA in putative mosaic 45,X/46XY subjects or to confirm the presence of a suspected Y fragment may assist in identifying subjects at risk for dysgenetic tumors. The utility of molecular probes for Y in this clinical situation will depend upon their potential to detect (1) small amounts of Y chromosome DNA in mosaic 45,X/46,XY subjects and (2) structurally altered Y chromosomes, especially del(Y)(q12). Four different but related experiments using a combination of Y DNA probes were designed to evaluate the extent to which these two variables involving sensitivity and specificity may affect the molecular identification of human Y DNA in mosaic 45,X/46,XY subjects.

Material and methods

General procedures

Plasmid isolation. The DNA probe pS4 (supplied by D. Hoar), which hybridizes to repetitive sequences on the Y chromosome, consists of a 850 bp *Pst* I nucleotide

Banding of Y				*4B-2*	
Heterochromatin	*Q-banding*	*Giemsa banding*	*Mbo I, pS4*	*EcoRI*	*Hind III*
	+	+	+	+	+
+					
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	+	+	+	+	+
+	−	−	+	+	+
+	+	+	+	+	+
−	−	−	−	+	+
−	−	−	−	+	+
−	−	−	−	+	+
?	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−
−	−	−	−	−	−

sequence derived from the 2.1 kb *Hae* III DNA fragment regionalized to the heterochromatic region of the long arm of the Y chromosome.[6] The probe 4B-2(supplied by K. Smith) is a recombinant phage construct containing a single copy 3.3 kb *Eco* RI fragment which maps to the proximal portion of the euchromatic region of the long arm of the Y chromosome. The recombinant plasmid was constructed from *Eco* RI digestion of the recombinant phage 4B-2 and insertion of the gel-purified fragment into the *Eco* RI site of pBR 322.[8] Both plasmids were propagated in *Escherichia coli* HB101. The bacteria were grown in Luria broth containing ampicillin. The plasmids were amplified with use of chloramphenicol and isolated according to the method of Ish-Horowitz et al.[9] The plasmids were digested with restriction enzymes, and the resulting fragments were purified through elecrophoresis on 0.8% low melting point agarose gel followed by purification with a NACS prepak column (Bethesda Research Laboratory) according to the manufacturer's recommendations.

DNA isolation. Total genomic DNA was isolated from 20 to 30 ml of ethylenediaminetetraaccetae–whole blood. The blood was mixed 1:1 with lysis buffer (1 mmol/L of magnesium chloride, 1 mmol/L of sodium phosphate (monobasic), ph 6.5, 0.8% Nonidet-P40, and 0.4% deoxycholate) and centrifuged in a JA20 rotor at 10,000 rpm for 30 minutes at 4° C. The pellet containing nuclei was dissolved in 10 ml of TEN (10 mmol/L of Tris, pH 8.3, 150 mmol/L of sodium chloride, and 5 mmol/L ethylenediaminetetraacetate), 3.5 ml of 5 mol/L sodium perchlorate, and 1.5 ml of 10% sodium dodecyl sulfate and digested for 1 hour at 65° C with proteinase K. The DNA was extracted twice with chloroform–isoamyl alcohol (24:1) and precipitated through the addition of ½ vol of 7.5 mol/L of ammonium acetate and 2 vol of absolute alcohol. The DNA was lypholyzed and subsequently dissolved in 1X TE plus 1X SSC (sodium citrate/sodium chloride), digested with pancreatic ribonuclease at 37° C for 30 minutes and redigested at 65° C for 1 hour with proteinase K. The DNA was then reextracted with chloroform-isoamyl alcohol and ethanol precipitated.

Restriction enzyme digestion, gel electrophoresis, and hy-

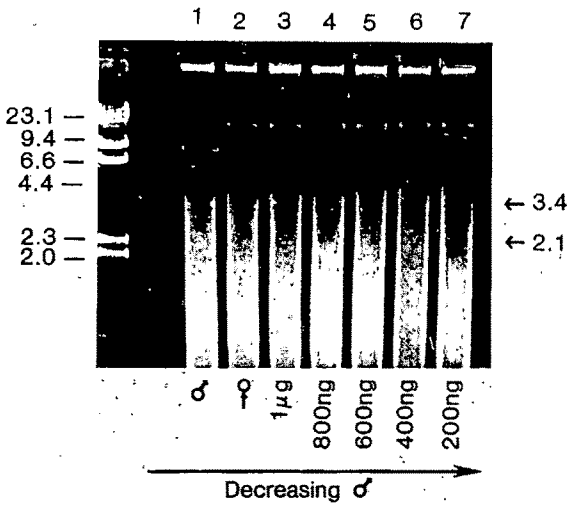


Fig. 1. *Hae* III restriction enzyme digests of normal male and female DNA, followed left to right by decreasing amounts of normal male DNA mixed with normal female DNA so that each lane has a total of 2 μg of the mixture. The 3.4 and 2.1 kb *Hae* III fragments are detectable down to 600 ng male to 1400 ng female DNA (30%) *(lane 5)*.

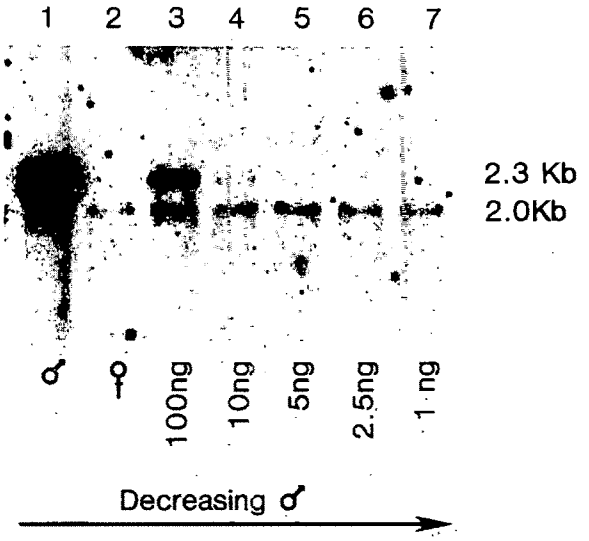


Fig. 2. A dilutional gel of *Mbo* I–digested DNA probed with pS4. *Lanes 1* and *2* represent normal male and 45,X female control samples. *Lane 3* represents a 1 μg mixture of 900 ng of 45,X DNA and 100 ng of normal male DNA. *Lanes 4* to 7 represent decreasing amounts of male DNA mixed with proportionately increasing amounts of 45,X DNA with maintenance of 1 μg of total DNA in each lane. The Y-specific 2.3 kb band is evident at the 5 ng concentration of male DNA, and possibly also at the 2.5 ng level. The 2.5 ng level represents a mixture of 2.5 ng/997.5 ng or 1 part male to 400 parts 45,X DNA.

bridization. Genomic DNA was digested with either *Hae* III, *Mbo* I, *Eco* RI, or *Hind* III under the conditions recommended by the supplier (BRL). The DNA was electrophoresed on 0.8% agarose gels and blotted with use of the method of Southern[10] to Gene Screen Plus filters (New England Nuclear). The filters were hybridized to either the pS4 or 4B-2 probes, which were phosphate 32 labeled by nick translation[11] and washed as recommended by the supplier. After the final wash the filters were washed for an additional 90 minutes at 65° C in 0.1X SSC with 1% sodium dodecyl sulfate.

Specific experiments

Experiment I: dilutional gel of male and female DNA digested with Hae *III.* Progressively smaller amounts of normal male DNA (2 μg to 200 ng) and normal female DNA were mixed to yield a final concentration of 2 μg. The mixtures were digested with *Hae* III and electrophoresed (2 μg/well) on a 0.8 agarose gel. After staining with ethidium bromide the gels were photographed on Kodak Technical Pan Film through orange, yellow, and ultraviolet contrast filters, under illumination with a short-wave length ultraviolet light (250 nm).

Experiment II: dilutional gel of male and female 45,X DNA digested with Hae *III or* Mbo *I and hybridized with pS4 plasmid.* Decreasing amounts of normal male DNA (100 ng to 10 pg) were mixed with female DNA from a 45,X gonadal dysgenesis subject, to a final concentration of 1 μg per lane, and digested separately with either *Hae* III or *Mbo* I. 1 μg each of the digested DNA dilutions was electrophoresed on a 0.8 agarose gel, blotted, and hybridized to a ^{32}P–labeled 850 bp *Pst* I fragment isolated from pS4.

Experiment III: Mbo *I digestion and hybridization with pS4 probe of DNA of study subjects.* The third experiment was designed to compare the specificity of molecular and cytogenetic techniques in the assessment of normal and altered Y chromosomes. Genomic DNA from all subjects (n = 20) and their fathers (n = 14) was digested with *Mbo* I, and 1.5 μg of DNA per well was electrophoresed on a 0.8% agarose gel, blotted, and hybridized to the pS4 probe as above.

CLINICAL SUBJECTS. All study subjects (n = 20), except one, were 45,X/46,XY mosaics with normal Y (n = 8) or variant Y (n = 12) chromosomes (Table I). In one of the 12 subjects with a variant Y chromosome a 45,X cell line was not detected (subject 9). The single cell line from this subject with an altered Y chromosome was obtained from amniotic fluid culture (maternal age indicated) at 16 weeks of gestation. The product of this pregnancy, now 3 months of age, is a normal-appearing male infant with bilateral scrotal testes. All other karyotypes (n = 19) were done on cells from peripheral blood.

The clinical phenotypes of the 20 subjects with normal and variant Y chromosomes ranged from phenotypic men with bilateral scrotal testes (n = 3) to women with Turner or quasi-Turner phenotypes (n = 17). The 17 phenotypic women had bilateral streak gonads (n = 12) or a unilateral streak gonad with a contralat-

eral testis (n = 5). Dysgenetic tumors occurred in three of the subjects. Two of the 17 phenotypic women had one or both rudimentary gonads replaced by a tumor. One patient (age 20 years) had a unilateral gonadoblastoma (subject 17) and the other at 15 years of age had bilateral tumors, dysgerinoma, and gonadoblastoma (subject 1). One enigmatic subject (No. 19) with a left intra-abdominal testis and right contralateral streak that were both extirpated at 5 years of age developed a tumor in a second right inguinal testis at the age of 15 years. The clinical and cytogenetic data are tabulated in Table I.

Cytogenetic analysis. Before DNA analysis all subjects were rekaryotyped in peripheral blood to update the percentage of mosaicism, to reclarify the structure of variant Y chromosomes, and to ensure that the molecular analysis was performed on the same sample from which the current percentage of mosaicism had been determined. The father of each proband, when available (n = 14), was karyotyped for comparison of Y structure. A minimum of 50 cells was counted, and five cells were karyotyped in all study samples. G-, Q-, and C-banding were performed on every study sample before DNA analysis on the same sample.

Based on the cytogenetic findings, the study subjects at this time were placed into three categories. Category A subjects had normal Y chromosomes by all cytologic parameters. Of category B subjects, one had a dicentric Yq and the other had a deleted Yq that eliminated Q-banding but preserved C-bands. Category C subjects were cytogenetically uniform in that they all appeared to have deletions of Yq that eliminated both Q- and C-bands (Table I).

Experiment IV: Eco *RI and*Hind *III digestion and hybridization with 4B-2 probe of DNA of study subjects.* To further categorize the deleted Y chromosomes (negative Q, negative C) in category C subjects, a fourth experiment was performed. Using normal men, women, and category A subjects as controls, all individuals in category C (n = 10) were further analyzed with the single copy DNA probe 4B-2. This probe, provisionally localized to the paracentric region of Yq, is proximal to the pS4 sequences.[8]

DNA from category A and C subjects was digested with either *Eco* RI or *Hind* III. The DNA was electrophoresed (7.5 μg per well) on 0.8% agarose gels, blotted, and hybridized to a ^{32}P-labeled 3.3 kb *Eco* RI insert isolated from the recombinant phage 4B-2 and subcloned into the *Eco* RI site of pBR 322.[8]

Results

Experiment I: dilutional gel of male and female DNA digested with *Hae* III. DNA from normal men cut with the restriction enzyme *Hae* III yielded two male-specific fragments. These fragments were 3.4 and 2.1 kb in size, and were not visible in *Hae* III–digested DNA from normal female controls (Fig. 1, lanes 1, 2).

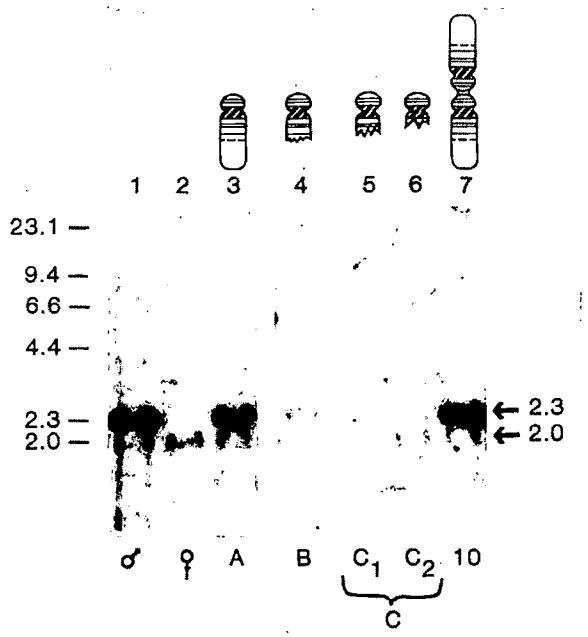


Fig. 3. An autoradiogram of total genomic DNA digested with *Mbo* I and probed with pS4. The lanes from left to right are as follows: *lane 1,* 1.5 μg of control normal male DNA; *lane 2,* 1.5 μg of control normal female DNA; *lane 3,* 1.5 μg of DNA from case 1, representative of cases 1 to 8 in the normal Y category (category A); *lane 4,* 600 ng (0.6 μg) of DNA from case 9, deleted of Q-bands but still positive for C-banding (category B); *lanes 5 and 6,* 1.5 μg of DNA from cases 11 and 19, representative of study subjects deleted of Q- and C-bands (category C); *lane 7,* 1.5 μg of DNA from subject with dicentric Y long arm.

In mixtures containing equal amounts of male and female DNA (1 μg/1 μg, lane 3) both of these bands were clearly visible on agarose gels. When female DNA was mixed with decreasing amounts of male DNA and electrophoresed on a gel, the bands were visible in photographs down to a concentration of 600 ng (30%) of total male DNA. With amounts of normal male DNA, less than 600 ng, these bands were not easily discernible (Fig. 1, lanes 3 through 7).

Experiment II: dilutional gel of male and female 45,X DNA digested with *Hae* III, *Mbo* I, and hybridized with pS4 plasmid. Normal male and female DNA restricted with *Hae* III and probed with pS4 exhibited a 2.1 kb band of hybridization. This single band, though less intense in female DNA, was present in both sexes. In DNA from controls and study subjects a distinct male-specific band was not evident with the *Hae* III digest and pS4 probe.

Normal male and 45,X female DNA restricted with *Mbo* I and probed with pS4 showed clear differences.[12] Male DNA, exhibiting tenfold more hybridization than female DNA demonstrated a 2.3 kb male-specific band.

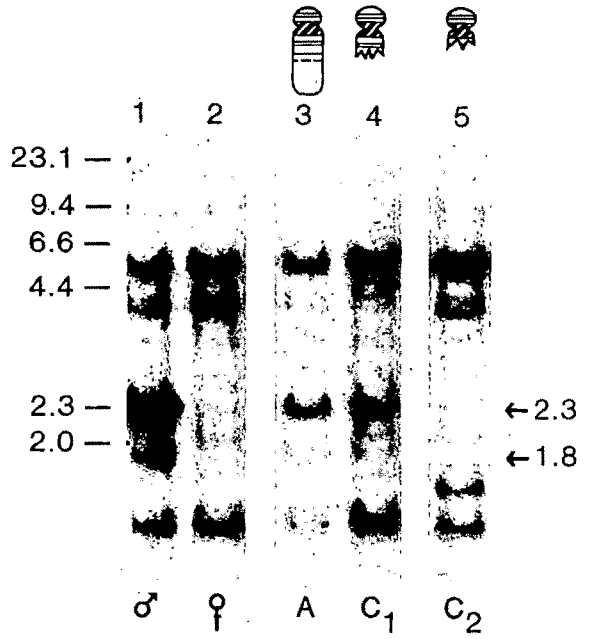


Fig. 4. Autoradiograms of total genomic DNA digested with *Hind* III followed by hybridization with 4B-2. *Lanes 1* and *2* represent normal male and normal female DNA. Normal male DNA after *Hind* III restriction has 2.3 and 1.8 kb fragments not present in female DNA. *Lane 3* represents one subject from category A with a morphologically normal Y chromosome. *Lane 4* is representative of subjects 11, 12, and 13 from category C deleted of Q- and C-bands, who hybridize with the 4B-2 probe and demonstrate the two male-specific fragments (category C_1). *Lane 5* is representative of the remaining seven subjects (14 to 20) in category C who do not hybridize with the 4B-2 probe (category C_2).

In gels containing 900 ng of 45,X DNA and 100 ng of male DNA this Y-specific 2.3 kb band was easily detected. This was visible down to an amount of 2.5 ng of male DNA. This represents a detection of 1 part male DNA in 400 parts 45,X DNA (Fig. 2).

Experiment III: *Mbo* I digestion and hybridization with pS4 probe of DNA of study subjects

Clinical subjects and cytogenetic analysis. The clinical and cytogenetic data in the 20 study subjects are tabulated in Table I.

CATEGORY A (N = 8). Subjects 1 thru 8, assigned to category A, and their fathers had morphologically normal Y chromosomes that were positive for Q-, G-, and C-bands. The percentage of 46,XY cells in peripheral blood ranged from a low of 10% (subject 1) to 94% (subject 8). Included in this category was one subject with bilateral dysgenetic tumors (No. 1), one subject with bilateral scrotal testes (No. 7), and two with unilateral testis and contralateral streak gonad (subjects 6 and 8).

CATEGORY B (N = 2). One subject (No. 9) was placed in this category because of his cytogenetic uniqueness. The morphology of the Y chromosome was intermediary between the normal Y (category A), and the morphologic variants seen in category C. The subject was a normal male phenotype initially ascertained in amniocyte culture, in whom a 45,X cell line had not been detected. The Y chromosome in the single cell line was completely negative for distal long arm Q-banding, but a distinct terminal heterochromatic band was evident with C-banding.

The second subject in category B (No. 10) had a dicentric Y chromosome, with loss of Yp and duplication of Yq, and was the only subject who was theoretically disomic for DNA on the long arm of the Y chromosome.

The fathers of both subjects (Nos. 9 and 10) in category B had cytologically normal Y chromosomes with distinct Q- and C-bands.

CATEGORY C (N = 10). Category C consisted of 10 subjects with clear morphologic deletions in their Y chromosome. All subjects in category C were negative for Q- and C-bands. The size of the Y was reduced in all subjects and in four subjects was simply a small minute centric fragment, acentric fragment, or ring (subjects 13, 14, 16 and 17). No further attempts with use of cytogenetic techniques were made to resolve the precise nature of these deleted chromosomes. The percentage of the variant Y cell line in category C subjects ranged from 14% (No. 11) to 92% (No. 13). The studied paternal Y chromosomes in category C were morphologically normal and positive for Q- and C-bands.

In this category were two subjects with tumors (Nos. 17 and 19), one azoospermic male with testicular failure and small bilateral scrotal testes (No. 20), and two subjects with unilateral testis and contralateral streak gonad (Nos. 15 and 19).

DNA analysis (Mbo *I, pS4).* The presence or absence of the 2.3 kb male-specific band is indicated for each individual subject in Table I. The autoradiographs, representative of each of the three cytogenetic categories, are seen in Fig. 3.

All subjects in category A with morphologically normal Y chromosomes had a distinct 2.3 kb band. This band was present in every category A subject (Nos. 1 to 8), even at the lowest level of XY mosaicism (10%) (Fig. 3, lane 3).

The subject in category B who was negative for Q-bands but positive for C-bands (No. 9) had the 2.3 kb male-specific band but at a reduced intensity (Fig. 3, lane 4). The subject with dicentric Yq (No. 10) exhibited the 2.3 kb band in increased intensity compared to a normal male control (Fig. 3, lane 7).

No subject in category C (Nos. 11 to 20) devoid of Q- and C-bands demonstrated the 2.3 kb band (Fig. 3, lanes 5, 6).

Experiment IV: *Eco* RI and *Hind* III digestion

and hybridization with 4B-2 probe of DNA of study subjects

DNA analysis. Digestion of normal male DNA with *Hind* III and blot hybridization with 4B-2 yields hybridization bands of 2.3 and 1.8 kb. These bands are not seen in autoradiograms of digested and blotted female DNA (Fig. 4).

Digestion of genomic DNA with *Eco* RI, and blot hybridization with the 4B-2 probe yields a single male-specific hybridization band of 3.3 kb. Normal female DNA does not demonstrate this band (Fig. 5).

The results of the 4B-2 probing after the *Hind* III and *Eco* RI restriction are tabulated for individual subjects in Table I and representative autoradiograms presented in Figs. 4 and 5.

Normal males and all category A subjects demonstrated the male-specific 2.3 kb, 1.8 kb *Hind* III, and 3.3 kb *Eco* RI fragments. Three of the 10 category C subjects (Nos. 11, 12 and 13) whose DNA failed to hybridize with the pS4 probe, hybridized with the 4B-2 single copy probe after both *Hind* III and *Eco* RI restriction. Representative autoradiograms from this group, referred to as category C_1, are depicted in Figs. 4 and 5 (lane 4). The remaining seven subjects in category C (Nos. 14 to 20) had DNA which failed to hybridize with the 4B-2 probe and were hence assigned to category C_2 (Figs. 4 and 5, lane 5).

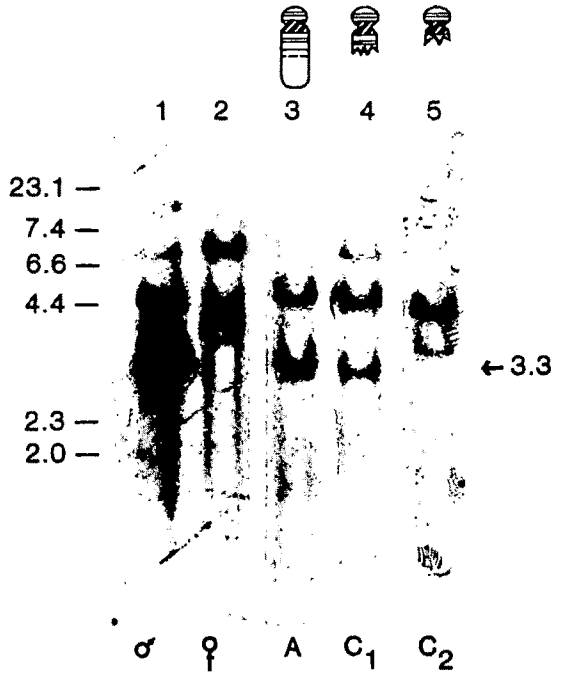


Fig. 5. Autoradiograms of total genomic DNA digested with *Eco* RI followed by hybridization with 4B-2. Male DNA *(lane 1)* has a 3.3 kb band not present in female DNA *(lane 2)*. Category A and C_1 subjects demonstrate this band *(lanes 3 and 4)*, but it is not seen in category C_2 subjects *(lane 5)*.

Comment

This series of four experiments addresses the ability of specific molecular techniques to identify human Y chromosome DNA. The clinical usefulness of these techniques will depend on their sensitivity and precision. The technique used in experiment I is quite practical but limited in sensitivity and precision. DNA extraction, digestion (*Hae* III), electrophoresis, viewing under ultraviolet light, and final analysis take approximately 2 days. By eliminating blotting and hybridization with a Y-specific probe, one shortens the time required for analysis but decreases sensitivity. This technique was not able to detect Y chromosome DNA in dilutional mixtures containing less than 30% normal male DNA. Conventional cytogenetic techniques, though more time consuming, have greater sensitivity. The precision of this technique (experiment I) is limited somewhat by the regional localization of the 3.4 and 2.1 kb repetitive fragments. These bands represent moderately repetitive DNA sequences interspersed throughout the long arm of the Y chromosome but located principally in the heterochromatic area. A deletion of Y (q12) eliminates the majority of these sequences and further restricts the precision and sensitivity of this technique. This abbreviated technique is nevertheless quite adequate for routine molecular sexing when nonmosaic 46,XY cell lines and morphologically normal Y chromosomes are the rule, rather than the exception.

Experiment II demonstrates that these problems of sensitivity and precision can be resolved in part by the use of Y-specific DNA probes. The male-specific repetitive DNA probe pS4, in mixtures of 45,X and 46,XY DNA, was able to detect down to 2.5 ng of male DNA. This probe detected male-specific DNA sequences except for the subjects with Yq deletions, which involved loss of both Q- and C-bands. The DNA from the 10 individuals with Y chromosome deletions proximal to the termination of C-banding (category C) did not hybridize with the pS4 probe. The one subject (No. 9) with a Yq deletion who demonstrated a distinct 2.3 kb Y-specific band still had a small distinct C-band on the Y long arm (category B). The *Mbo* I, pS4 2.3 kb band in this subject, devoid of Q-bands but positive for C-banding, suggests that the sequences corresponding to the pS4 probe are defined on Yq by the upper limits of C-banding and are not restricted to the Q positive region. The molecular data, contrary to general cytogenetic opinion, suggest that the chromosomal regions representing the C- and Q-bands do not overlap in their entirety. C-heterochromatin, as defined by the upper limits of the pS4 sequences, seems to extend more proximal than Q-banding.

Experiment III clearly defines the limits of the pS4

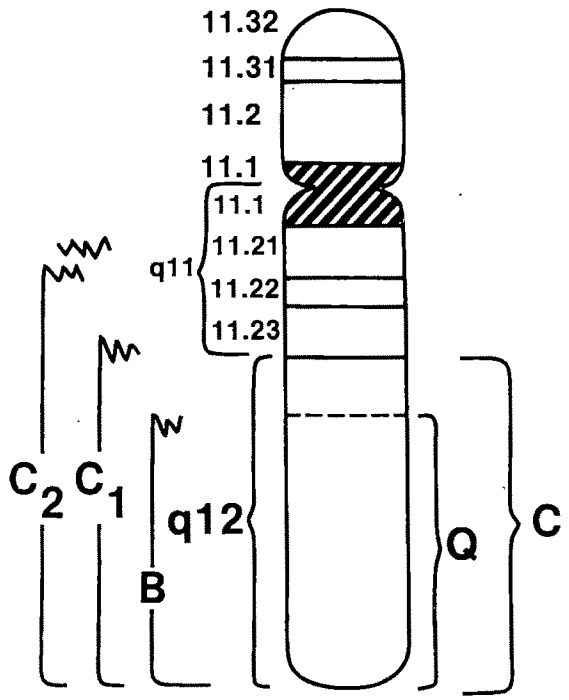


Fig. 6. A schematic representation of the extent of the deleted Yq in each of the three categories of Y variants is compared to the normal category A. In variant B the breakpoint extends to the top of the Q-band but does not reach to the top of the C-band. Type B variant, similar to a cytogenetically normal Y (category A), hybridizes with both the pS4 and the 4B-2 probes. Type C_1 variant is indistinguishable from type C_2 variant cytogenetically. Both variants are devoid of Q- and C-bands. Type C_1 does not hybridize with pS4 but does hybridize with 4B-2, indicating a more extensive deletion than variant type B. Variant type C_2 does not hybridize with either probe and is presumed to have the most extensive deletion into the proximal portion of the long arm of Y, or alternatively to be isochromosomes for Yp with complete absence of long arm DNA.

sequences in this study group. A 2.3 Kb male-specific band is not seen in any of the 10 subjects with Yq deletions who have lost both C- and Q-bands (category C). One may assume that these 10 individuals have a Y deletion extending up into the euchromatic portion of the long arm of Y.

In experiment IV it is apparent that even the 10 individuals in category C who appear to be cytogenetically indistinguishable are not homogeneous on DNA analysis. The DNA from three of these subjects, when restricted with *Eco* RI and *Hind* III, hybridize with the more proximally located single copy 4B-2 (category C_1). The DNA of the remaining seven subjects, with presumably more extensive Y chromosome deletions, did not demonstrate the male-specific bands with this single copy probe (category C_2). This sugggests that the largest DNA deletions are present in these seven individuals. who are more or less cytogenetically indistinct from the other three del(Y) subjects (Fig. 6). Alternatively some of these seven subjects may have complete absence of Yq DNA. The severe variant chromosomes in these subjects may be isochromosomes for Yp or even represent fragments of X.

It is apparent from these four experiments that cytogenetic techniques will continue to be necessary to define the gross morphologic structure of variant Y chromosomes. DNA techniques can then be selectively used to more precisely characterize the limits of these alterations (Fig. 6).

Characterizing the DNA composition of the variant or altered Y chromosome may provide insight into the critical determinants for oncogenesis and gonadal differentiation. Sensitive and precise techniques to detect covert Y chromosome DNA, which are capable of identifying variant Y chromosome DNA, will help to define the population of individuals at risk for dysgenetic tumors and may provide clinical insight into their variant gonadal development. The widespread application of DNA technology to detect all variant Y chromosomes will require centromeric-specific Y probes.[13]

We gratefully acknowledge Kirby Smith and David Hoar for providing the pS4 and 4B-2 DNA probes. We thank David Muram, Sid Wilroy, and John Phillips for providing blood samples. This work constitutes publication 10 of the Human Genetics Institute, Medical College of Georgia.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Gloria E. Sarto, Milwaukee, Wisconsin. From the time of the first descriptions of the human sex chromosomes, efforts to ellucidate the mechanism of sex determination have been directed toward the cytogenetic/phenotypic investigations of individuals with aberrant sexual differentiation. From these studies and others it is clear that genes in several parts of the genome are involved in the developmental process of both male and female sexual development. Though not the focus of this research, the study of structurally abnormal sex chromosomes indicates that specific genetic information on the Y chromosome is responsible for maleness and genetic information on the X chromosome is necessary for normal ovarian function.

The Y chromosome is one of the smallest human chromosomes; it comprises 0.5 to 1.0% of the total diploid amount of genetic material. Cytogenetic staining techniques indicate that a large portion of the Y chromosome is heterochromatic. Also, the Y chromosome is practically devoid of functional genes except those dealing with male sex determination—located on the short arm of the Y (Y_p)—and possibly those factors controlling spermatogenesis, located on the nonfluorescent part of the long arm of Y (distal Yq11).[1] Fifty percent of the Y is composed of repeated DNA sequences; however, efforts are being directed toward characterizing the human Y chromosome with respect to its single copy DNA content and function, which is the focus of this research.

Initially, before banding techniques, only large structural chromosome abnormalities could be discerned. With conventional banding and later with high resolution banding, the ability to pick up more subtle anomalies improved; however, the introduction of recombinant DNA technology to the field of genetics has proved to be an even more valuable tool. The haploid DNA content of the human genome is 3×10^9 bp. With around 400 bands detectable in the haploid complement, one can calculate that one band spans about 10^7 bp or about 10,000 kb. The techniques outlined and used by the authors allow them to identify target DNA fragments down to 2.3 to 3.3 kb, obviously a far greater resolution.

The purpose of the proposed study was to determine the sensitivity and the specificity of two DNA probes, pS4 and 4B-2, in individuals with normal and structurally abnormal Y chromosomes. The authors showed that the technique is useful in detecting small amounts of DNA. They proved that the DNA probes used are specific for Y chromosomes, that is, they are not detected in normal females and in fact are specific for the long arm of the Y (Yq). The 4B-2 sequence has been localized by Burk et al.[2] to be between Yq11 and Y chromosome subcentromeric region. The authors placed the sequence on the euchromatic region of the long arm of Y (Yq11), which may be a bit more distal than the localization stated by Burk et al. This is possible as the Yq11 band is quite broad. The authors state that of 10 individuals negative for both the distal long arm Q- and C-bands, three (category C_1) hybridized to the 4B-2 probe and seven (category C_2) did not. Recognizing the fact that even with prophase banding there are not a great number of bands on the euchromatic portion of the long arm of the Y chromosome, I would still like to ask if the authors attempted at all to use prophase banding on the cases in category C, to more clearly delineate where the break on the Y chromosomes occurred and consequently define more precisely where the 4B-2 sequence is localized.

Given the sensitivity of DNA probes, I would like to ask the authors what use this method would have in prenatal fetal sexing. Do they think it may be possible to use this technique to detect male fetuses, possibly from fetal cells circulating in the maternal peripheral circulation. Also, I would like them to comment on the possible uses of these DNA probes for localizing other factors on the Y such as those controlling spermatogenesis, presumably in Yq11, or perhaps the tendency for tumor development in 45,X/46,XY mosaics to particular portions of the chromosome.

In summary, the authors have shown, as have others, the usefulness of recombinant DNA techniques in clinical medicine. Specific DNA probes can be used to identify fragments of the Y not identifiable by cytogenetic methods. With the development of more probes localized to various positions along the Y chromosome, discrimination between other deletions like those noted in categories C_2 and C_1 will be possible. More important perhaps, particularly with respect to the Y chromosome, will be the ability to delineate the exact position of the male sex-determining genes on the short arm.

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Dr. S. Elias, Chicago, Illinois. I would also like to compliment Dr. McDonough and his colleagues on a

most interesting and informative piece of work. I have two questions for Dr. McDonough.

You indicated that Y-specific probes could prove useful in providing some insight into the predisposition of neoplasia in individuals with gonadal dysgenesis and a Y chromosome. Two groups of patients known to be at increased risk are phenotypic females with X-linked recessive 46,XY gonadal dysgenesis as well as sex-reserved males with nonmosaic 46,XX complements. Have you had the opportunity to use your two DNA probes to study such individuals?

Second, although it appears to be a very complex story now, HY antigen is still considered to be somehow associated with testicular determinant(s). In your study group, have you tried to correlate the results from either of the Y-specific probes with the presence or the absence of HY antigen? This would be of particular interest in the subgroup with the Y structural abnormalities.

Dr. Paul G. McDonough (closing). I will respond to Dr. Sarto's comments and questions and also to Dr. Elias' questions.

First, I would like to discuss the possible resolution between cytogenetics and molecular genetics. Imaging the gap between cytogenetics and molecular genetics is not easy. Resolution differences between the two are measured in millions of base pairs compared to a single base pair change. A specific single copy gene may be 800 to 1200 bp, whereas an undefined cloned DNA segment may be 10 to 20 db, or 20,000 bp. An average chromosome like the X has 1×10^8 bp. The banding patterns on X give markers that are about 5×10^6 bp apart. In situ hybridization technology is capable of identifying gene sites in bands and subbands of stained prometaphase chromosomes. However, the level of resolution even with this technique within a subband may span 5×10^3 to 10^4 kb. Into this gap you are assigning a single gene of 100 bp, or an undefined cloned sequence ranging in size from 10 to 20 kb. At the most you are dropping 20 kb into a 5000 kb gap.

A subband on the human Y may have an even wider resolution span than 5×10^3-10^4 kb. The Y chromosome studied with medium resolution banding (350 bands per genome) has six bands. This number increases only to nine or 10 with high resolution banding (1000 bands). Ellen Magenis at the University of Oregon has some high-resolution studies of the Y in which the idiogram reveals up to 15 bands. In general the small size of the Y and the infrequency of rearrangement has limited *site-specific* mapping by in situ hybridization and translocation mapping with use of human-mouse cell hybrids. However, as more genes are isolated and cloned it will become important to map them to a specific site on a chromosome, and ultimately to a specific nucleotide sequence on a chromosome. At that point we will have bridged the gap between cytogenetics and molecular genetics.

Regarding the second question, location of 4B-2 sequences (DYS 15) were thought initially to be closer to the Y centromere and in the region of the "spermatogenesis factor" described by Tiepolo and Zuffardi,[1] as indicated by Dr. Sarto. Our work and that of others suggest that the 4B-2 sequences are clearly in the Yq11 region but distal to both the actin (ACT 2) and argininosuccinate synthetase (ASSP6) pseudogene sequences on the Y long arm. Recently Wolfe et al.[2] have been able to isolate a centromeric repeat family of DNA sequences that have Y specificity (centromeric Y-alphoid repeats). These sequences in the Y centromeric region are able to detect Y chromosome DNA down to a concentration of 50 ng of male DNA. Their centromeric location makes these sequences ideal for identifying tiny chromosomal fragments as Y chromosome DNA.

The third question, regarding detection of male DNA in maternal blood, is difficult for me to answer because I do not know exactly the number of fetal cells that one might find in the maternal circulation in an average pregnancy. As I indicated earlier, the repetitive copy pS4 probe will detect 2.5 ng of normal male DNA in 997.5 ng of normal female DNA. This represents 1 part male DNA to 400 parts female DNA with 1 μg of total DNA in each lane or well. If one increased the DNA in the lane to 10 μg, then one should be able to increase the sensitivity to 1 part male DNA in 4000 parts female DNA. If each cell has approximately 6 to 10 pg of DNA, then this roughly equates as one cell in 4000. There is a limit to the sensitivity of the probe because ≈50 copies of the pS4 sequences are located on autosomes compared to ≈500 copies on the human Y. As one increases the total concentration of DNA, the 2.3 kb band may begin to appear in situations in which the DNA is completely 46XX, due to these 50 autosomal sequences.

Second, the pS4 probe will not detect Y chromosomes that are deleted of C-bands.

The 4B-2 sequences are single copy, unique to the Y chromosome. The only technical limitations of this probe are the requirement for a larger sample of DNA, usually 7 to 10 μg in each lane, and the Y chromosome must have the 4B-2 sequences.

The centromeric Y-alphoid sequences, as I have indicated, will detect down to 50 ng of male DNA in a sample not containing other genomic DNA. One of the practical clinical limitations of the alphoid Y-specific family may be that 20,000 copies are present in the rest of the genome. An *Eco* RI 5.5 kb fragment appears to be specific for the Y-alphoid repeat family. However, if female and male DNA were mixed together similar to our dilutional experiments with pS4 there is some concern that autosomal DNA may obfuscate the 5.5 *Eco* RI band. This experiment has not yet been done but would be of obvious interest to the obstetrician.

It is possible that the detection of male DNA in fetal blood may lose some of its clinical significance in the years to come. DNA probes in the form of restriction fragment linked polymorphisms are available for the two most common X-linked disorders—hemophilia (Factor VIII deficiency) and Duchenne's muscular dystrophy. Patients may prefer molecular analysis of am-

niocytes for definitive diagnosis of the male fetus at risk.

In reply to the question about whether we do prophase chromosome studies, all of our patients and their available fathers (12 of 20) were karyotyped by means of G-, C-, and Q-banding. We experimented with distamycin in some of our 10 subjects with acentric fragments (n = 4) and one subject (No. 19) with a tiny metacentric Y suspicious for 45X/46,X, i (Yp). The latter was not helpful, so we moved to our molecular analysis once we had repeated our cell counts for coordinating the molecular results.

The small size of the Y chromosome has made karyotypic definition of translocations and deletions difficult. In fact, the small size of the Y limiting the cytogenetic definition of rearrangements and the absence of extensive recombination has hindered the regional localization of genes and gene sequences on the Y. The transition from conventional karyotyping at 250 to 350 bands to high-resolution chromosome analysis at 1200 bands adds only 4 or 5 new bands to the Y. The patients in category C (C_1 and C_2) would be candidates for high-resolution chromosome analysis. However, in the absence of some reference band or bands we felt that high-resolution chromosome analysis would not clarify the character of these minute fragments. At the time we optimistically thought that our DNA probes would do this for us. As it turned out, seven of the 10 variant chromosomes in category C subjects (category C_2) remain undiagnosed. We are waiting for our centromeric Y-specific probe, but we would be happy to forward the cell lines to you for high-resolution chromosome analysis.

The fifth question concerned whether part of the Y chromosome was related to oncogenesis and testicular determinant genes, what part of the Y is oncogenic, and whether this area or gene sequences *overlap* with testicular determining genes. This is difficult to answer. It is perhaps easier to answer the question of overlap versus exclusivity first. On the basis of phenotype-karyotype-tumor correlations, it would appear that gene sequences encoding tumor are on many occasions distinct from gene sequences encoding for testicular differentiation. The 45,X/46,XY patient without testis but with *bilateral rudimentary streaks* who develops a tumor must not have de facto functional genes for testicular differentiation. Magenis and her group in Oregon describe a phenotype female 46,X,del (Y) (p 11.2 → pter) who developed a gonadobastoma in place of one of her rudimentary streak gonads.[3] The area Y p 11.2 → pter is presumed to contain the "critical" testicular determinant genes. This child, though lacking these sequences, developed a tumor. Although oncogenesis may be mediated through sequences other than testicular determinant genes, no oncogene has been identified on the human Y chromosome. The closest oncogene is a human cellular homolog of the Harvey murine sarcoma virus, HRAS2, which has been tentatively assigned to the long arm of X. The presence of this oncogene on X reminds us that the localization of genes for testicular differentiation and the mechanism through which they encode for testis is still unresolved. The majority of investigators continue to pursue the classical approach that "critical genes for testicular differentiation" are encoded and transcribed from the Y chromosome itself. The majority of the available molecular data obtained from studies of 46,XX males, 46,XX true hermophrodites, and 46,XY gonadal dysgenesis suggests that this approach should and seems to be rewarding.

A minority group of investigators, impressed by the lack of Y transcripts, have turned their attention to the X chromosome. Their theories have been bolstered by the discovery of gene sequences in the heterogametic-banded Krait snake (BKM sequences), which are important in sexual differentiation and have been conserved in every species up through man. The only startling finding by Metzger et al.[4] in the Erickson group at Ann Arbor is that these same sequences in man are not on the Y chromosome but on the X. These data suggest that the Y chromosome in man may play only a regulatory role, and active transcripts producing gene products important in sexual differentiation may be coming from the BKM sequences on the maternal derived X.

Our group in Augusta under Jay Tischfield has begun to isolate mRNA from early stages of human development, in order to determine by Northern blotting whether hybridization to our previously described Y probes occurs. Clear hybridization should suggest that Y DNA is actively transcribing during early development, if not in the adult. If hybridization does not occur, then one may need to look for transcripts that hybridize with the BKM sequences from the X chromosome depending upon which theory for sexual differentiation you advocate.

I would now like to respond to the questions of Dr. Sherman Elias. He asked whether we have studied any X-linked or sporadic 46,XY gonadal dysgenesis patients. To date we have studied seven patients with sporadic 46,XY gonadal dysgenesis (Swyer's syndrome). However, the DNA probes that we have used all involve DNA sequences localized to the long arm of the Y chromosome. DNA from these seven patients reveals normal male-specific bands with pS4, 4B-2, and more recently the argininosuccinate synthetase sequences. The latter, symbolized as ASSP6, are pseudogene sequences located more proximal and closer to the centromere on Yq than the 4B-2 and pS4 sequences. We and others have avoided the 46,XY gonadal dysgenesis subjects where vertical transmission with X-linked inheritance is suspect from pedigree analysis. The suspicion in these familial cases is more directed at an X-linked gene, and one may not anticipate any Y DNA abnormalities.

The critical deletions or mutations in 46,XY gonadal dysgenesis are probably on Yp. David Page at the Whitehead Research Institute in Boston has identified a deletion of certain cloned Y sequences in three of four 46,XY gonadal subjects studied, and interestingly

enough, the same deleted sequences are the ones present in 46,XX sex-reversed males. In approximately 75% of 46,XX males studied to date hybridization with single copy short arm Y probes has been demonstrated. Single copy Y DNA has also been identified in a 46,XX true hermaphrodite. The quest is obviously for a sequence or family of sequences that are critical for normal sexual differentiation.

We did not do any H-Y antigen studies in our patients. This area remains so controversial that results are difficult to interpret. In fact, the Y chromosome is singularly lacking in known gene products. Recently an antibody 12E7 reacting with a cell surface antigen has been identified, and the antigen appears to be a Y gene product. The gene itself is referred to as MIC2Y. The lack of other gene products to date has created some concern or skepticism as to whether such products actually exist. On the other hand, many cloned sequences have been mapped to Y but without any specific function or gene products.

I believe that your interest in the molecular basis for sporadic 46,XY gonadal dysgenesis is a very relevant one. These individuals as studied by most investigat have cytologically normal Y chromoso nes. Since th gonadal phenotype is bilateral rudimentary streak nads, they would be apt candidates for a molecu deletion involving the critical testicular determin genes as Page has found in a small series of patien

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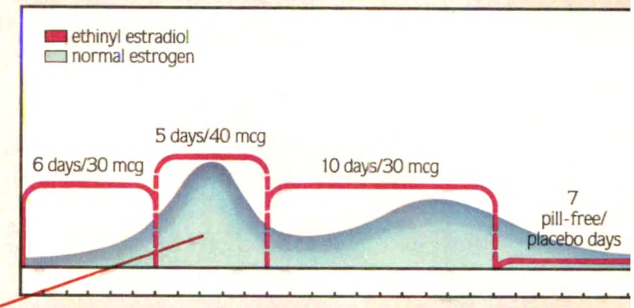
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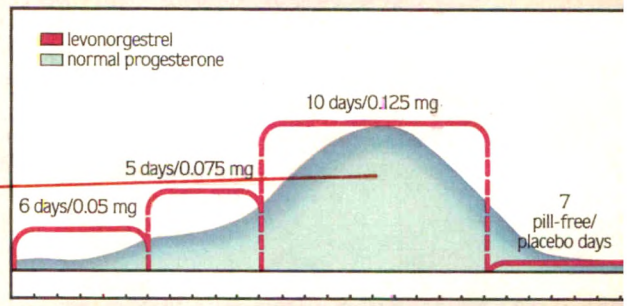
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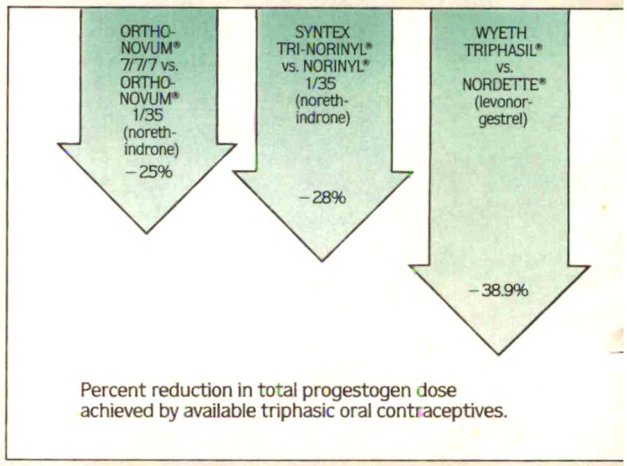
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HARVENGT[3]	6	⇄	⇄	⇄	⇄
SKOUBY[4]	6	⇄	↑	⇄	⇄
MÄRZ[5]	3	⇄	↑	⇄	⇄
BRIGGS[6]	18	⇄	↑	⇄	
CULLBERG[7]	6	⇄	⇄	⇄	
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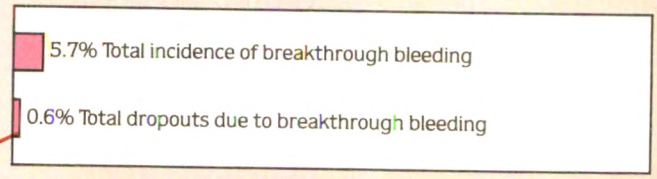
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See important information on last page.

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Indications and Usage—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly ot types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, fo any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of fi missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becomin pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinician recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed wh on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in use increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in t 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously ha had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infa has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not l prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depressio recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administere with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woma becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecti the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenyto sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (se Warnings):thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatmen edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation wh given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergi mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepeni intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the associa has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, change appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multifo erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directe and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medicatio or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

Triphasil® Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

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Partial purification of relaxin from human seminal plasma

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Human seminal plasma contains approximately 50 ng/ml of relaxin (specific activity = 1.3 ng/mg protein). During preliminary purification, semen plasma was delipidated, rehydrated, and loaded onto small octadecyl silica columns that were eluted with a TEAF/acetonitrile gradient system. Results were monitored by radioimmunoassay. The resultant partially purified human relaxin demonstrated biologic activity in the rat uterine segment bioassay. Nine liters of semen plasma was delipidated, rehydrated in TEAF, and subjected to high-performance liquid chromatography by a step gradient followed by a linear gradient. The active eluate was further purified by ion exchange chromatography. Pooled recovery fractions provided a total of 45.8 μg of relaxin. An aliquot flash evaporated and desalted by gel filtration chromatography provided 1.85 μg of relaxin in 25.2 mg protein, specific activity 73.4. This material is being used as immunogen in the production of antihuman relaxin antibodies by monoclonal technique. Our procedure represents the first and only successful partial purification of human relaxin to yield sufficient quantity and purity for antibody production. (Am J Obstet Gynecol 1986;154:749-55.)

Key words: Relaxin, semen, HPLC

Relaxin was discovered in 1926 by Hisaw.[1] It was shown to be a hormone that modifies the maternal reproductive tract during pregnancy. The major source of relaxin for experimental use is porcine ovaries, structures particularly rich in relaxin and readily available from abattoirs. Since porcine relaxin is devoid of tyrosine and histidine, amino acids necessary for iodination, development of a radioimunoassay (RIA) was delayed until a technique was developed to radiolabel the molecule.[2] This allowed several laboratories to establish RIAs for relaxin. The structure of porcine relaxin was simultaneously determined by two laboratories.[3, 4]

In 1976 our laboratory clearly showed that relaxin is a human hormone of pregnancy, produced by the corpus luteum.[5] The primary structure of human relaxin, as determined by recombinant DNA technology, significantly differs from that of porcine relaxin.[6] Human relaxin has never been purified from a biologic source. This is because its concentration is relatively low, even in the corpus luteum, the richest source in humans. Starting material for purification is difficult to obtain. The hormone is a small molecular weight peptide of approximately 6000 daltons. It is lipophilic and has a high isolectric point. These characteristics result in its property of adhering to laboratory ware and other proteins. For example, we have determined that partially purified human relaxin adheres to and will not elute from certain kinds of filters. Its adherence to other substances prevents its clean precipitation by ammonium sulfate after dilipidation. Rather, it precipitates in a very broad peak with some of the hormone precipitating in all concentrations of ammonium sulfate. Similar problems occur when attempts are made to precipitate relaxin by graded ethanol concentrations after delipidation.

In 1980 immunoreactive relaxin was determined to be present in human semen plasma.[7] We confirmed this observation and demonstrated the seminal relaxin to be biologically active in the guinea pig pubic symphysis palpation assay.[8] Relaxin concentration in semen plasma averages 50 ng/ml compared with approximately 1 ng/ml in serum from pregnant women. Since semen plasma is plentiful compared with other human relaxin sources, we could use this as a starting material for purification and accept low yields caused by the molecule's properties. Our strategy is to isolate and purify human semen relaxin to a level of purity sufficient to produce monoclonal antibodies to human relaxin, monitoring purification with both RIA and bioassay. The antibody can then be used to further purify human relaxin by affinity chromatography. The purified relaxin as well as the antibodies could then be used as research tools. In this report we described the partial purification of human semen relaxin to be used as an immunogen.

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Material and methods

Semen plasma. Semen plasma for starting material to be used in relaxin purification was obtained from a fertility laboratory. Semen was collected by masturbation after 3 to 5 days of abstinence. The samples were allowed to stand at room temperature for approximately 3 hours for completion of semen analysis. Samples were then centrifuged at 600 × g for 10 minutes, the pellet was discarded, and the semen plasma was pooled and stored frozen at −20° C.

Assays. Relaxin was measured by RIA with the use of rabbit antiporcine relaxin antibody R6, developed by O'Byrne and Steinetz.[9] ^{125}I polytyrosylated porcine relaxin was used as ligand and purified porcine relaxin (CMa') as standard. All results are expressed as nanograms per milliliter of immunoreactive equivalents of porcine relaxin. The sensitivity of this assay is 50 to 100 pg per tube. The interassay coefficient of variation is 12.9% and intraassay coefficient of variation is 6.1%. The average precision of the assay is 13.9%. All samples were measured in triplicate.

Protein was determined by the method of Lowry et al.[10]

All glassware was silanized with Prosil-28 (SCM Chemicals, Gainesville, Florida) before use.

The rat uterine horn segment bioassay for relaxin with in vivo estrogen and progesterone pretreatment has been described in detail.[11] Immature female Long-Evans rats were treated subcutaneously with 1 μg of estradiol benzoate in sesame oil for 3 days. Two milligrams of progesterone in sesame oil was injected subcutaneously on days 2 and 3. The animals were killed on day 4 and their uteri isolated. Uterine horn segments were mounted in a double-jacketed organ bath attached to an isometric smooth muscle transducer. Electrodes from a direct-current stimulator were positioned so that one was free in the bath and the other touched the uterine horn segment. The muscle was bathed in 5 ml of modified Locke solution. Spontaneous contraction patterns were observed and a baseline contraction pattern was established with electric stimulation of 5 V, 60 Hz, for 5 seconds every minute. After contraction patterns were established the bath was changed and 5 ml of modified Locke solution containing the substance to be tested was added. Baseline and stimulated contractions were then monitored. At the end of the experiment the uterine segment was washed in modified Locke solution alone three times and contractions were again observed to demonstrate that inhibition of contractions was not a nonspecific toxic effect.

Delipidation. The first step in all purification protocols was delipidation. Semen plasma was extracted with 10 vol of cold acetone. The supernatant was decanted and discarded. The precipitate was then diluted with 10 original vol of hexane and the supernatant discarded. The precipitate was then extracted with 10 vol of acetone and saved by suction filtration on Whatman No. 541 filter paper. The precipitate was air dried overnight at room temperature and dissolved in deionized water. The insoluble material was removed by centrifugation at 1000 × g for 10 minutes. The supernatant was lyophilized and stored at −20° C. In the large-scale purification scheme, the acetone wash was acidified with 0.1N HCl. All reagents used in purification were high-performance liquid chromatoagraphic (HPLC) grade.

Octadecyl silica (ODS) purification—small scale. The powdered delipidated semen plasma from 100 ml of semen plasma was dissolved in 500 ml of 0.25N TEAF, pH 3.0. This was divided into 29 parts and loaded on disposable Sep-Pak ODS columns, 191 mg powder/17.2 ml/column. The columns had been previously activated with 10 ml of methanol and equilibrated with 10 ml of 0.25N TEAF, pH 3.0. The eluate was recirculated and reloaded for a total of 10 times to ensure complete adsorbance to the column. After loading, each Sep-Pak was washed with 10 ml of TEAF. This was followed by a wash with 10 ml of 20% acetonitrile (CH_3CN)/80% TEAF. This was followed by a wash with 9.5 ml of 40% CH_3CN/60% TEAF. Most of the relaxin eluted in the last 9 ml of this wash. The corresponding fractions from all Sep-Paks were combined and assayed for relaxin.

HPLC was carried out on a Supelcosil semipreparatory (10 mm by 25 cm) ODS column containing 5 μm of particles with 10 nm of pores (catalog No. 5-8358, Supelco Inc., Belafonte, Pennsylvania). The chromatograph used was a Beckman model 324-MP advanced gradient liquid chromatograph with two solvent delivery pumps and a CRT controller (Beckman Instruments, Inc., Fullerton, California). The column effluent was monitored at 254 nm with a Hitachi variable wavelength spectrophotometer, model 155-10, equipped with a high-pressure flow cell. The recording was made on a Kipp & Zonen dual pen strip chart recorder (Kipp & Zonen, Bohemia, New York) set on two absorbance units full scale, with a chart speed of 0.5 cm/min. Fractions were collected at 1-minute intervals with a Gilson model 201 fraction collector (Gilson Medical Electronics, Inc., Middleton, Wisconsin) into borosilicate glass tubes.

The column was equilibrated with 12% CH_3CN/TEAF. The pooled Sep-Pak fractions were diluted with 2 vol of TEAF to restore CH_3CN to 12% a concentration at which relaxin completely adsorbs to the column. Sample loading was accomplished via the aqueous solvent delivery pump. This eliminates the need for drying the Sep-Pak fraction, rehydrating, and clarifying the solution, steps that result in loss of relaxin. After loading, the pump and columns were flushed with 100 ml of 12% CH_3CN/TEAF followed by 100% TEAF.

Table I. Partial purification of relaxin from 100 ml of human semen plasma

Procedure	*Total activity (ng)**	*Protein (mg)†*	*Specific activity (ng/mg)*	*Yield (%)*	*Purification (fold)*
Original semen plasma	8860	4343	2.0	100	(1.0)
Acetone, hexane extracted semen plasma	5150	1525	3.4	58.1	1.7
Sep-Pak eluate	1690	36.5	46.3	19.1	23.2
HPLC eluate	1450	22.5	64.4	16.4	32.2

*Total activity by RIA.

†Total protein determined by the method of Lowry et al.[10]

Relaxin was eluted by means of a linear gradient of CH_3CN/TEAF, increasing CH_3CN from 12% to 22% in the first minute. In the next 21 minutes the CH_3CN was increased to 50%. Flow rate was 4.73 ml/min. Fractions were collected in borosilicate glass test tubes (12 by 75 mm, RTU; Becton Dickinson, Parsippany, N.J.). Relaxin eluted at 77.5 ml. Relaxin was measured by RIA directly, aliquoting the mixture into 0.5 ml of RIA buffer (0.01 mol/L phosphate-buffered saline solution with 1% ovalbumin, pH 7.0).

Large-scale purification. Nine thousand ninety-four milliliters of pooled semen plasma containing 461.5 μg of immunoactive relaxin and 343.1 gm of protein was extracted with acid acetone, hexane, and acid acetone. The precipitate was dried and rehydrated with 0.25N TEAF, pH 3.0. This was clarified by ultracentrification at 40,000 rpm (250,000 × g) for 1 hour.

Preparatory octadecylsilyl silica high-pressure liquid chromatography. The Prep LC/System 500 (Waters Associates, Milford, Massachusetts) with ODS Prep-Pak 500 cartridge, 5.7 by 30 cm, was activated with 4 L of methanol and equilibrated with 4 L of TEAF. The delipidated semen plasma was loaded onto the Prep-Pak and recirculated for 8 hours at a flow rate of 250 ml/min. Elution by a step gradient proceeded at a flow rate of 100 ml/min. Four liters of 0.25N TEAF, pH 3.0, was used as the initial wash. The second wash was 6 L of 20% CH_3CN/80% TEAF. The next wash was 8 L of 40% CH_3CN/60% TEAF, the first 5 L collected in 500 ml fractions and the remaining 3 L collected as one fraction. A final wash of 4.5 L of 60% CH_3CN/40% TEAF was performed.

Fraction 2 of the 40% CH_3CN/60% TEAF step gradient, containing 53% of the load and 80% of the total recovery, was filtered on a 0.45 μm pore size hydrophilic Millipore Durapore membrane (No. HVLP04700, Millipore/Continental Water Systems, Bedford, Massachusetts) to remove precipitated protein that formed during storage at −20° C. The solution was diluted to 4 L in 0.25N TEAF, pH 3.0. The Prep-Pak was equilibrated with 4 L of 5% CH_3CN/95% TEAF, loaded with the diluted fraction 2 and recirculated for 30 minutes. A linear gradient of 10% to 50% CH_3CN/30 minutes was chosen. Fractions were collected, with fraction 1 containing 970 ml, fractions 2 through 25 containing approximately 250 ml each, and fraction 26 containing 828 ml. Fractions 13 through 17 were pooled, with a total volume of 1278 ml.

Ion exchange chromatography. After preliminary study, 1041 ml of the pooled fractions 13 through 17 were flash evaporated to 83 ml, ionic strength 0.2 mol/L. This was diluted fivefold with water and the pH adjusted from 3.0 to 5.0 by dropwise addition of triethylamine to a volume of 415 ml, ionic concentration 0.093 mol/L. During pH adjustment, a precipitate formed that was removed by ultrafiltration at 250,000 × g for 60 minutes. The supernatant had a pH of 5.0, ionic strength of 0.1 mol/L, and a volume of 405 ml. There was no measurable loss of relaxin in the precipitate.

Carboxymethyl Fractogel (Fractogel TSK CM-G50-S Merck Chemical Division, Rathway, New Jersey) was equilibrated in 0.05 mol/L ammonium acetate, pH 5.0, and packed in a Pharmacia K 50/30 glass column (5 by 15 cm, bed volume 294.5 ml) fitted with Pharmacia A-50 flow adaptors at both ends (Pharmacia Fine Chemicals, Piscataway, New Jersey). Three hundred ninety-five milliliters was pumped onto the column with a Pharmacia P-4 peristaltic pump, flow rate 2 ml/min. Relaxin was not detected in the column effluent. Unadsorbed proteins were washed off with 0.05 mol/L ammonium acetate, pH 5.0. Relaxin was eluted from the column with a 0 to 1 mol/L NaCl linear gradient in 0.05 mol/L ammonium acetate, pH 5.0. Three-minute fractions (6.1 ml) were collected. After 99 fractions were collected, baseline optical density at 280 nm was not reached. Elution was continued until 26 additional samples were collected and the baseline had reached its pregradient elution level. Relaxin was detectable in the last fraction indicating that some relaxin remained on the column. Elution was continued isocratically with 1 mol/L NaCl in 0.1 mol/L of ammonium acetate, pH 5.0, for another 75 fractions. Relaxin-containing fractions were combined into eight separate pools.

Gel filtration chromatography. Size exclusion chromatography used Sephadex G-50 (fine) packed in a Pharmacia K-26/100 glass column (2.6 by 100 cm; bed

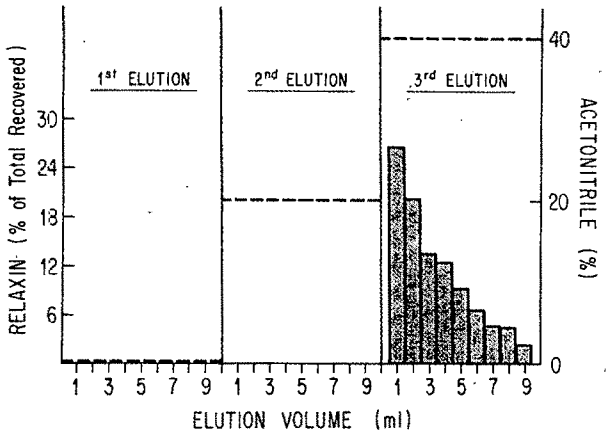


Fig. 1. Triple-step gradient of CH_3CN/TEAF used in the elution of human semen relaxin from a Sep-Pak.

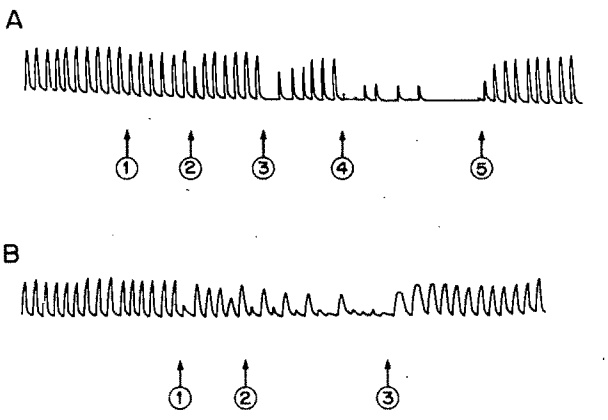


Fig. 2. Effect of relaxin on electrically stimulated rat uterine horn segments. In *A*, 2 ng of porcine relaxin (NIH-R-P1) is added at *arrows 1-4*. *Arrow 5* indicates a wash. In *B*, 8.8 ng of partially purified human semen relaxin is added at *arrows 1 and 2*. *Arrow 3* indicates a wash.

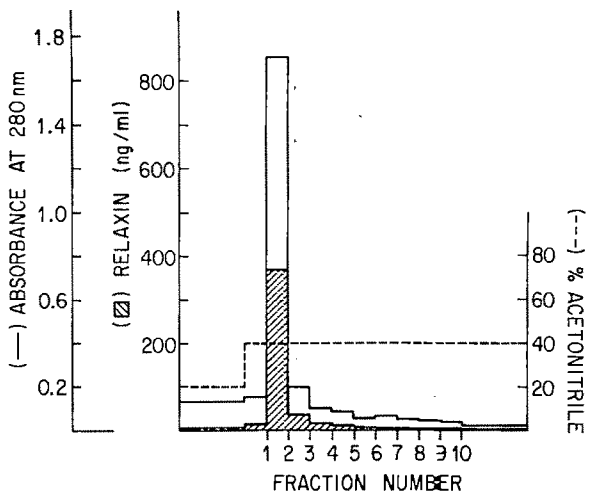


Fig. 3. Multiple-step gradient elution of human semen relaxin from a Prep-Pak with CH_3CN/TEAF. The Prep-Pak load was solubilized delipidated powder from 9094 ml of human semen plasma.

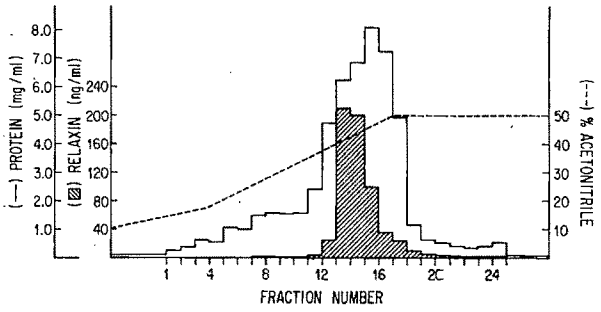


Fig. 4. Rechromatography by a linear gradient of CH_3CN/TEAF on the Prep-Pak of fraction 2 from the step gradient (Fig. 3).

volume 530 ml) fitted on top with a Pharmacia A-26 flow adaptor. The column was equilibrated in 0.01 mol/L ammonium acetate, pH 5.0, at a flow rate of 2 ml/min. All fractions were collected at 4-minute intervals in 13 by 100 mm borosilicate glass RTU test tubes. The column was presaturated with 0.1% bovine serum albumin. The load solution, 20.85 ml, flash evaporated from 468 ml taken from a preliminary recovery from ion exchange chromatography, contained 5.06 μg of relaxin, with 221.8 mg of protein, specific activity 22.82 ng/mg.

Results

Small-scale purification. The results of the small-scale purification of 100 ml of semen plasma can be seen in Table I. The results of the step-gradient elution of relaxin from a Sep-Pak can be seen in Fig. 1. No relaxin is eluted by the 20% CH_3CN/80% TEAF wash. Most of the relaxin is eluted in the 40% CH_3CN/60% TEAF wash.

The pooled mobile phase from the relaxin eluate from HPLC was evaporated under vacuum and lyophilized four times to completely eliminate all solvent. This material was rehydrated and applied to the rat uterine horn segment relaxin bioassay. As can be seen in Fig. 2, human seminal relaxin purified by these methods is active in this assay. The high sensitivity of this assay compared with other bioassays of relaxin allows its use in the monitoring of purification of human semen relaxin.

Large-scale purification. After delipidation, the pooled semen plasma contained 437.9 μg of relaxin. This was chromatographed by Prep-Pak HPLC by a step gradient. The results are seen in Fig. 3. Fraction 2 contained 232.3 μg of relaxin/11.1 gm protein, specific activity 20.93 ng/mg.

The results of Prep-Pak HPLC with a linear gradient are seen in Fig. 4. The load of relaxin was 204.6 μg. Fractions 13 through 17 contained 198.8 μg of relaxin and 8.95 gm of protein, specific activity 22.21 ng/mg.

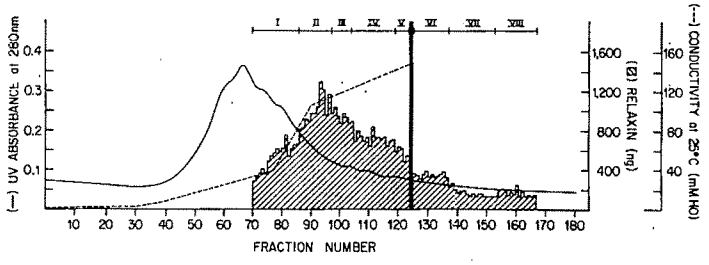


Fig. 5. Chromatography of Prep-Pak purified human semen plasma by ion exchange chromatog raphy on carboxymethyl Fractogel TSK with the use of an NaCl gradient. The *vertical arrow* indicates where the second session of column elution started. *Roman numerals* signify pooled fractions.

The results of ion exchange chromatography of 1041 ml of pooled fractions 13 through 17 from HPLC are shown in Fig. 5. Recovery of fractions 70 through 124 contain 37.82 μg of relaxin and 0.737 gm of protein, specific activity 51.32 ng/mg. The purest pool was VIII, containing 1.79 μg of relaxin/5.95 mg of protein, specific activity 301 ng/mg. This is a 13.5-fold increase in purity over that of the column load. If pools III through VIII were arithmetically combined, the relaxin content of this pool would be 29.1 μg of relaxin/138.74 mg of protein, specific activity 210.9 ng/mg. The estimated total recovery, if the entire quantity of starting material had been pumped through all of the steps, would be 10.67%.

Gel filtration chromatography. The desalting procedure was run with the eluate of 468 ml from pooled recovery fractions 57 through 135 of a preliminary ion exchange chromatography run. These results can be seen in Fig. 6. Pool II contains 1.85 μg of relaxin and 25.2 mg of protein, specific activity 73.4 ng/mg, with an ionic strength of 0.01 mol/L. Pool III contained 0.458 ng of relaxin/1.04 μg of protein, specific activity 440.4 ng/mg. However, this pool had an ionic strength of 0.43 mol/L, indicating that it was not salt free. Pool II is being used for monoclonal antibody production.

Comment

To our knowledge, this article reports the first successful partial purification of human relaxin from a biologic source in sufficient quantity and purity for antibody production. Our attempts at purification of human seminal relaxin have provided several useful techniques to decrease procedural losses. These include keeping the relaxin in acidic solution and silanization to decrease adherence to labware. Avoidance of drying and filtering partially purified relaxin, but rather maintaining it in a dissolved state, also helps decrease procedural losses. For example, in an earlier purification protocol we filtered relaxin on a Millex GV filter, a polyvinylidene difluoride hydrophilic membrane contained in a disposable polystyrene holder. We first tested the filter with porcine relaxin and it allowed passage without loss. However, this filter unit retained 75%

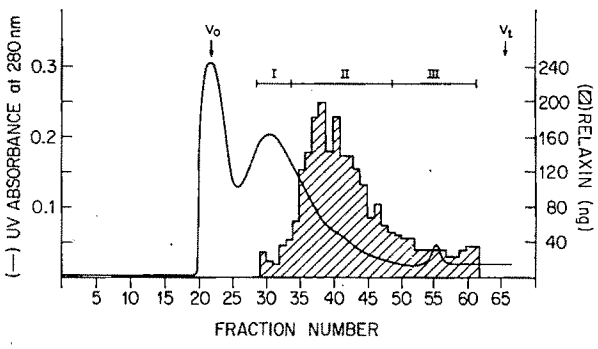


Fig. 6. Desalting of CM Fractogel TSK–purified human semen plasma on a Sephadex G-50 (fine) column. *Roman numerals* indicate pooled fractions. *Vo*, void volume; *Vt*, total volume.

of the human relaxin. We were unable to elute this relaxin by any technique.

Our procedures will provide experience in devising techniques for the purification of relaxin-like peptides from other sources, some with much lower concentration or availability.

It is likely that there will be considerable microheterogeneity in our relaxin preparation. Semen plasma contains many proteolytic enzymes such as amino and carboxypeptidases, which may partially degrade the native hormone but may not affect its biologic activity. Porcine relaxin can accept signifigant loss of the C-terminus of the B chain without loss of biologic activity.[12]

Hudson et al.[6] have shown that there are two genes for human relaxin as identified from a human genomic library. These two genes encode markedly different relaxin peptides. Little is known of the expression of these genes except that one is preferentially expressed by human ovaries during pregnancy. Characterization of seminal relaxin is necessary to determine which gene is expressed in the male. It is tempting to postulate that there is divergent gene expression in the male and female. This possibility can be evaluated by purification of the seminal hormone and comparison of its structure to that of ovarian relaxin. The studies may provide models for the evaluation of the hormonal regulation of gene expression.

The partial purification of relaxin described herein

will provide material for the production of antihuman relaxin antibodies, which, in turn, can be used for final purification of seminal relaxin as well as establishment of human relaxin immunoassays. Antirelaxin antibodies will also be useful tools in determination of the biologic roles of relaxin.

Because of significant primary structural differences, administration of nonhuman (porcine) relaxin to humans has the potential danger of being immunogenic. Human relaxin in sufficient quantities for clinical use will be available only as material generated by recombinant DNA methodology. However, use of the recombinant DNA–derived relaxin will require verification of the structure of the native human hormone.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. KAMRAN S. MOGHISSI, Detroit, Michigan. Relaxin was discovered almost 60 years ago. It is a polypeptide hormone with a similar structural identity to that of insulin and nerve growth factor. Its main source appears to be the corpus luteum of pregnancy, but it is also produced by the male prostate gland and is found in many target tissues such as cervix, myometrium, decidua, and breast connective tissue. Its principal mechanism of action appears to be the facilitation of remodeling of connective tissue in target organs to allow the necessary changes in organ structure during pregnancy and parturition. Additionally, in some mammals, relaxin inhibits myometrial contractility until near the end of pregnancy.

Possible roles for relaxin in the human include enhancement of sperm motility and penetration into cervical mucus, ovum penetration, implantation of blastocyst, uterine stromal remodeling during pregnancy, the inhibition of premature labor, cervical ripening, and collagen biosynthesis in disorders of collagen metabolism such as scleroderma and arthritis.[1,2]

Porcine relaxin has been well studied and purified. This preparation has been used in most animal experiments and some clinical studies.

The amino acid sequence of a human relaxin derived from the recent identification of a genomic clone for relaxin is now known.[3] Approximately 50% homology between porcine relaxin and this human relaxin exists. Porcine and human relaxins share the same tertiary structure and some of the same amino acid sequences at biologically active sites.

Human relaxin has not, as yet, been purified and the development of a specific assay must await the availability of purified samples of human relaxin for antibody development.

The stated goals of Dr. Weiss and his associates are as follows: (1) purification of relaxin to a sufficient degree so that workable amounts of relaxin-specific antibodies can be obtained, (2) use of the antirelaxin antibodies to prepare affinity immunosorbants that can be used to complete the relaxin purification, (3) sequence of the purified relaxin to determine molecular structure, and (4) use of recombinant DNA methods to batch up adequate amounts of relaxin for clinical studies.

The work presented here deals with only the first item, namely partial purification. The purification of relaxin from seminal plasma is a difficult problem and Dr. Weiss and his colleagues should be complimented for their persistance and their ability to collect "9 L" of semen as their starting material. However, there are severe limitations in the experimental approach used by the authors.

First, the levels of immunoreactive relaxin in human seminal plasma are exceedingly low, approximately 9 μg per 9 L (representing 4300 μg of proteins). Even assuming purification to homogeneity with 100% yield, which would require a 500,000-fold enrichment, the amount of relaxin recovered would be barely sufficient for structural analysis. Dr. Weiss reports a 32-fold purification with only 16% yield after three purification steps. This is certainly not an encouraging result.

Second, the authors plan to use the partially purified relaxin, which contains only 0.006% of total protein as immunoreactive relaxin, as immunogen for an attempt to raise monoclonal antibodies to relaxin. What are the chances of success of this approach? This manuscript contains no data regarding this but I heard from Dr. Weiss that he has been successful in raising antibodies to relaxin. Another question is how does he intend to screen for clones secreting antibodies to relaxin? There is no guarantee that an immunoaffinity resin with an antirelaxin monoclonal antibody would yield relaxin in sufficient yield or purity for structural studies.

Despite these problems, Dr. Weiss and his colleagues should be encouraged to pursue these studies with the hope that their success might lead to the availability of human relaxin and precise identification of its many biologic functions.

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Dr. Willard M. Allen, Glenwood, Maryland. People ask me what I do nowadays, and I say I celebrate anniversaries. Actually, the word "progesterone" celebrates its fiftieth anniversary this year. The name relaxin goes back, I suppose, about 10 years more.

When progesterone came on the scene, the majority of scientists thought that relaxin had been buried, thrown away as it were. However, I knew Fred Hisaw very well. As a matter of fact, with one of his previous graduate students in Rochester, I repeated some of his original work including the bioassay, which was performed by the measurement of palpation, really of separation of the symphysis in the guinea pig.

If you are interested in bioassay, this is probably one of the most treacherous of all. The truth of the matter is, though, the reason for Hisaw getting at this originally was that the guinea pig has fetuses that are so large at birth that they could never be born at all unless the symphysis relaxed. The symphysis really does relax to the point where you can actually almost slip an index finger between the two sides of the symphysis. That is real relaxation, and it makes possible the delivery of the fetuses.

Now, we hear about porcine relaxin. Actually, the first relaxin Hisaw ever had came from the corpora lutea of pigs. We called them sows in those days. Nowadays the language gets more elaborate.

Relaxin has never died, and it is really comforting after all these years to know that it is still a hormone that was sidetracked on the road to progesterone. Of course, in Hisaw's laboratory as well as in the Corner-Allen Laboratory in Rochester, the relaxin went down the drain because the steroids that we were both after were not in the fraction that contained relaxin. In fact, Hisaw and Fevold did obtain progesterone a little later than we did. But the fact still remains that nothing in the way of ovarian hormone combinations, that is, estrogens and progesterone, ever produced relaxation of the symphysis of the guinea pig such as Hisaw observed. His original extracts were made with acid alcohol of pigs' corpora lutea. This method extracted both progesterone and relaxin; the relaxin was lost in the purification that finally yielded pure progesterone.

I guess the mystery now is not that there is a hormone but what it does.

Dr. Weiss (Closing). The concentration of relaxin is certainly low in semen, but it is richer in semen plasma than in any other material except for the corpus luteum. The concentration of relaxin in semen plasma was (as stated in the article) 461 μg/9 L. The corpus luteum is relatively inaccessible so there really is no choice but this for determining the structure of the native hormone.

Our yields are low, 10% at the end of the purification to date. The reason for this, of course, is the physical properties of relaxin, making it rather difficult to deal with.

However, we have been successful, as I mentioned, in producing monoclonal antibodies, and the monoclonal antibody should be useful for performing a final purification step on the affinity column.

Another question that was asked was, "Will there be enough for a structural analysis?" If by structural analysis, you mean starting from scratch, separating the chains, and doing a total analysis of both chains, the answer is likely no, but that is not necessary. There are two putative relaxin structures encoded by genes identified from a genomic library. These two putative structures are remarkably different. What would be necessary is simply to take the small amount of hormone that will be available, digest it proteolytically, and essentially "fingerprint" it, determine the structure of small areas, and then compare it with gene A or gene B to determine which gene is expressed in the male. The gene that is expressed in the corpus luteum is known, so by this methodology it certainly is possible to be successful in determining the structure of the male relaxin, determining if it is similar or different from the female relaxin, and deriving appropriate material for further study. If necessary, we probably will have enough material to even immunize a rabbit for polyclonal antibody production. We now have 30 μg of the partially purified material stockpiled for further work. We also have stockpiled another 10 L of human semen, so with what we have learned it is possible to simplify the purification now that there will be an affinity column and provide the material that we need.

Human ovarian oxytocin: Its source and relationship to steroid hormones

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Chicago, Illinois

To determine the site of oxytocin in human ovaries and its relationship with ovarian steroids, oxytocin and steroid hormones were measured in ovarian tissues, ovarian vein, and peripheral blood. Corpus luteum had significantly higher oxytocin, estrone, estradiol, and progesterone concentrations than corpus albicans and ovarian stroma ($p = <0.01$ to <0.001). Oxytocin concentrations in corpus luteum correlated significantly with estrone, estradiol, and progesterone. Oxytocin in corpus luteum increased from 14.0 ± 1.8 ng/gm of wet weight in early to 30.8 ± 0.9 ng/gm in midluteal phases ($p = <0.001$). Reverse phase high pressure liquid chromatography showed similarity between oxytocin in corpus luteum and synthetic oxytocin. Ovarian vein draining corpus luteum had significantly higher plasma oxytocin (11.8 ± 1.5 pg/ml) than those without corpus luteum (2.1 ± 0.2 pg/ml) or in the peripheral blood (2.9 ± 0.3 pg/ml) ($p = <0.001$). Oxytocin in corpus luteum correlated significantly with its ipsilateral ovarian vein level of oxytocin, estrone, progesterone, and 17α-hydroxyprogesterone. Our findings demonstrate that oxytocin is present and probably produced in corpus luteum and secreted into its ovarian vein; it may regulate corpus luteum release of progesterone, 17α-hydroxyprogesterone, and estrone. (Am J Obstet Gynecol 1986;154:756-63.)

Key words: Oxytocin, corpus luteum, estrogen, progesterone, luteolysis, luteotropic

In 1910 Ott and Scott[1] discovered the oxytocic action of the corpus luteum when they observed that its extract increased the quantity of milk. This interesting observation was not pursued further until this decade when oxytocin was identified in several species.[2] In primates, oxytocin or an oxytocin-like peptide was shown to be present in human ovaries by two independent laboratories,[3,4] which was later confirmed.[5] Oxytocin was also found to be present in the monkey corpus luteum.[6] The role of ovarian oxytocin on ovarian physiology is not established but based on in vitro studies; postulated functions include both a luteotrophic and luteolytic action.[7] Although cultured bovine[8] and ovine[9] luteal cells have been shown to synthesize oxytocin in vitro, there is no information available on the exact source of oxytocin in the human ovary. Furthermore, the in vivo relationship between ovarian oxytocin and ovarian steroid hormones has not been examined. Therefore a study was carried out to examine ovarian oxytocin and steroid hormones. The objectives of the study were to determine (1) the presence and concentrations of oxytocin or an oxytocin-like peptide in different types of normal human ovarian tissues, (2) the relationship between oxytocin and estrone, estradiol, and progesterone in corpus luteum, and (3) the relationship between corpus luteum oxytocin and oxytocin, estrone, estradiol, progesterone, and 17α-hydroxyprogesterone in the ovarian and peripheral venous blood.

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Material and methods

Collection of ovarian tissues. Ovarian tissues were obtained from women undergoing gynecologic operations and who gave consent. The tissues were obtained from the pathologist immediately upon their removal. The decision to remove the ovaries was made exclusively by the surgeon operating on the patient, based on clinical considerations. Sixty-nine ovaries were obtained from 45 premenopausal women who had known last menstrual periods. Women from whom fresh corpora lutea were obtained had regular cycles, and the day of their luteal phase was further established with endometrial dating by the pathologist. To minimize prolonged ischemia of the ovary while hysterectomy was proceeding, the ovary was removed separately at the beginning of the surgical procedure. The ovaries were kept on ice and immediately transported to the surgical pathology laboratory for pathologic examination, where they were weighed and specimens taken for histologic study. After this, the ovaries were dissected to obtain corpus luteum, corpus albicans when available, and ovarian stroma. Thirteen fresh corpora lutea, 18 corpora albicantia, and 49 ovarian stromal

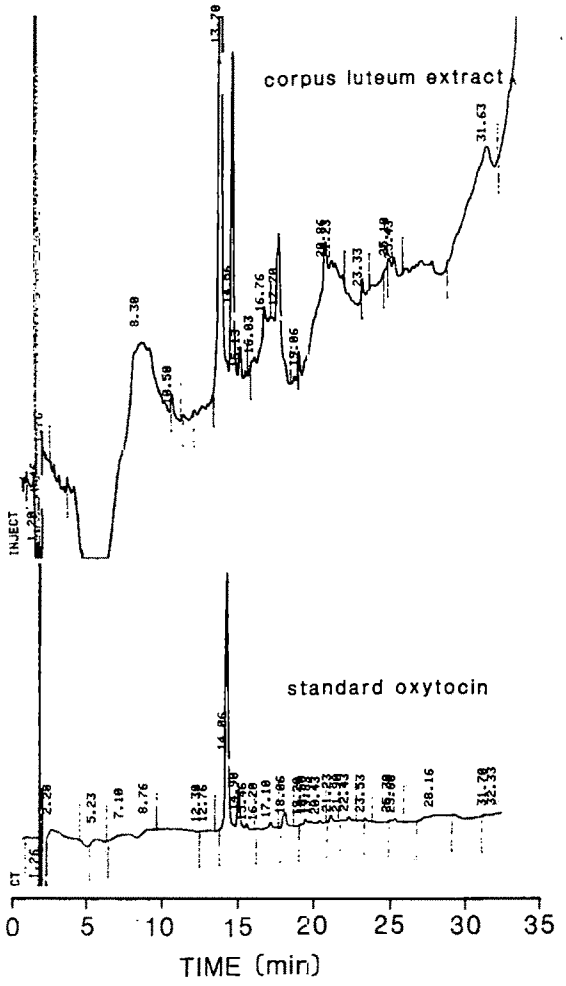


Fig. 1. Reverse-phase high pressure liquid chromatogram of an extract of human corpus luteum (day 24 of the menstrual cycle) compared to standard oxytocin (Sandoz). The samples were chromatographed at room temperature on a Bondapak C_{18} (3.9 mm × 30 cm) column with use of a Waters binary automatic gradient controller (model 680) and the protein peaks detected by a fixed wavelength ultraviolet detector (model 441). The solvent systems used were A (99.9% water and 0.1% trifluoroacetic acid) and B (70% acetonitrile, 29.9% water, and 0.1% trifluoroacetic acid). The gradient used was 2 minutes of 35% B, 37 minutes of 60% B, and 65 minutes of 100% B. A peak corresponding to the standard oxytocin peak is seen with the corpus luteum extract. The retention times of this peak of the corpus luteum extract as well as of standard oxytocin were 14.06 minutes.

tissue samples were available for the study. The tissues were further identified and confirmed histologically. The separated tissue samples were weighed, finely minced in a dish kept cold on crushed ice, homogenized in 0.4 mol/L of acetic acid at 4° C (1 ml of acetic acid per 50 mg of tissue) and centrifuged at 800 × g for 20 minutes. The supernatant was separated, lyophilized, and kept at −20° C until ready for further purification and oxytocin assay.

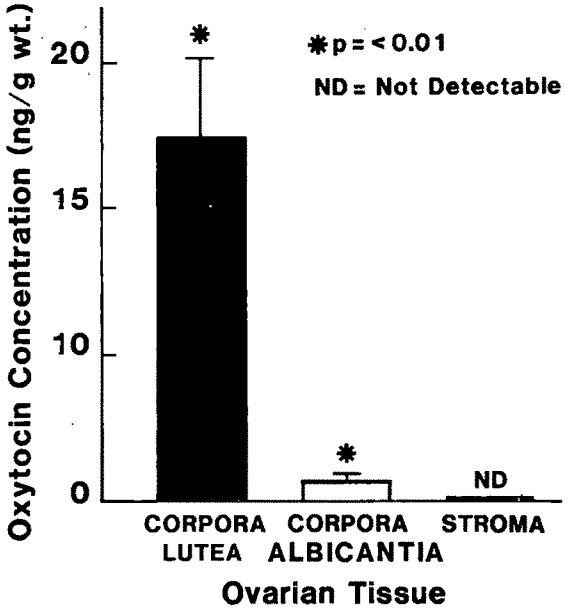


Fig. 2. Mean ± SE concentrations of oxytocin in human corpora lutea, corpora albicantia, and ovarian stroma. Corpora lutea had significantly higher oxytocin concentrations than corpora albicantia (p = <0.01). Ovarian stroma had no detectable oxytocin.

Blood collection. Peripheral venous blood, from the antecubital vein and ovarian venous blood samples, were collected into chilled heparinized vacutainers. When the ovary was to be removed, the infundibulopelvic ligament was isolated and the ovarian vein blood was drawn by venipuncture with use of a No. 19 gauge butterfly needle just before the ligament was clamped. Preliminary studies carried out on oxytocin levels in peripheral venous plasma obtained before premedication and general anesthesia and while under general anesthesia showed no significant effect of the latter on plasma oxytocin. Thus all peripheral blood samples were collected simultaneously with the respective ovarian vein sample. There were eight ovarian vein blood samples draining a corpus luteum, 24 ovarian vein blood samples from ovaries without corpus luteum (10 in the follicular and 14 in the luteal phase), and 28 antecubital vein blood samples (13 in the follicular and 15 in the luteal phase). The blood samples were kept cold, immediately transported to the laboratory in crushed ice, and centrifuged at 4° C to separate the plasma. An aliquot of the plasma was acidified with 0.2 ml of 1N hydrochloric acid for each milliliter of plasma and frozen at −70° C until ready for oxytocin assay. The remaining plasma was also frozen at −70° C for sex steroid hormone assay.

Oxytocin assay. The lyophilized extract from the ovarian tissue was resuspended in 0.4 mol/L acetic acid and passed through an octadecasilicyl silica (Sep-Pak)

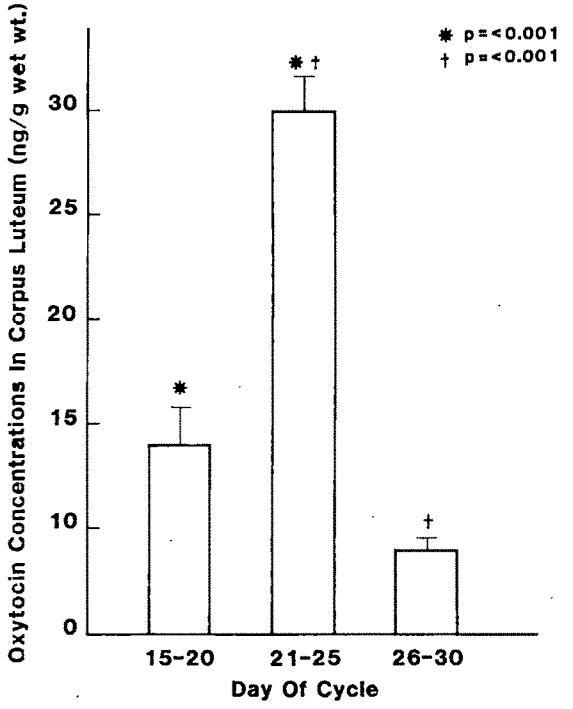


Fig. 3. Mean ± SE oxytocin concentrations in human corpus luteum during early (days 15 to 20), mid (21 to 25), and late (26 to 30) luteal phases. There was a significant increase in oxytocin concentration from the early to the midluteal phase (p = <0.001), followed by a significant decrease in the late luteal phase (p = <0.001).

column to remove impurities.[10] The eluates were collected, lyophilized, and resuspended in 1 ml of oxytocin radioimmunoassay (Tris–hydrochloric acid) buffer; an aliquot of this was taken for radioimmunoassay of oxytocin. Recovery of oxytocin from the ovarian tissue was determined by adding known quantities of iodine 125–labeled oxytocin to the minced tissue, which was then homogenized in 0.4 mol/L of acetic acid at 4° C and centrifuged at 800 × g for 20 minutes, and an aliquot of the supernatant was taken for determination of radioactivity. The mean ± SE (standard error of the mean) recovery of oxytocin for the extraction of oxytocin from the tissue was 91.9% ± 0.9%. All results of tissue concentrations of oxytocin were expressed without correction for recovery.

The acidified plasma was extracted for oxytocin with Fuller's earth as described by Dawood et al.[11] Immunoreactive oxytocin was determined by a specific and sensitive radioimmunoassay as described by Dawood et al.[11] with the use of ^{125}I-labeled oxytocin and a specific oxytocin antiserum raised in rabbits immunized against oxytocin conjugated to bovine serum albumin. The sensitivity of the assay was 1 pg per assay tube (1 μL of oxytocin = 2 pg) or 0.2 ng of oxytocin per gram of tissue, with intraassay and interassay coefficients of variation of 7% to 15% and 12% to 18% respectively. The cross-reaction of the oxytocin antiserum has been shown to be <0.8% with angiotensin and <0.2% with lysine-vasopressin, arginine vasopressin, vasotocin, luteinizing hormone releasing hormone, neurophysins I and II, and α-melanocyte-stimulating hormone. The final recovery of oxytocin in plasma with use of our method of extraction with fuller's earth was 42.5% ± 0.5%.[11] All results for plasma oxytocin were corrected for procedural losses.

Serial dilutions of the lyophilized, Sep-Pak–purified ovarian tissue extract in 0.05 mol/L of Tris-hydrochloric acid buffer containing 1% bovine serum albumin were subjected to radioimmunoassay of oxytocin to determine parallelism with the oxytocin standard curve. Reverse-phase high pressure liquid chromatography of the corpus luteum extracts was performed on a Waters binary automatic gradient controller (model 680), and the protein peaks were detected with use of a fixed wavelength ultraviolet detector (model 441). The samples were chromatographed at room temperature on a Bondapak C_{18} (3.9 mm × 30 cm) column with use of two different solvent systems, A (99.9% water and 0.1% trifluoroacetic acid) and B (70% acetonitrile, 29.9% water, and 0.1% trifluoroacetic acid). The gradient employed was 2 minutes of 35% B, 37 minutes of 60% B, and 65 minutes of 100% B.

Steroid hormone assay. To determine ovarian tissue concentrations of estrone, estradiol, and progesterone, a small sample of the tissue was finely minced, homogenized in Tris-glycerol buffer at 4° C as previously described,[12] and centrifuged at 800 × g for 20 minutes, and the supernatant was used for extracting the steroid hormone. The ovarian tissue extract and the plasma samples were individually extracted with double-distilled anhydrous diethyl ether. The ether extract was evaporated to dryness, resuspended in phosphate buffer, and chromatographed on Sephadex-LH_{20} to separate estrone and estradiol, and progesterone from 17α-hydroxyprogesterone.[13, 14] Estrone, estradiol, progesterone, and 17α-hydroxyprogesterone concentrations were determined by specific radioimmunoassays as previously described.[13, 14] The specific antibodies used were raised against estrone-6-oxime bovine serum albumin conjugate, estradiol-6-oxime bovine serum albumin conjugate, progesterone-11-oxime bovine serum albumin conjugate, and 17α-hydroxyprogesterone 7α-carboxyethyl thioether bovine serum albumin conjugate for estrone, estradiol, progesterone, and 17α-hydroxyprogesterone, respectively. All results were corrected for procedural losses.

Statistical analysis. Data were expressed as mean ± SE. Means were compared by the nonpaired Student's *t* test or a one-way analysis of variance with the use of a two-tailed p value. Paired samples of ovarian vein plasma and corpus luteum hormone concentrations

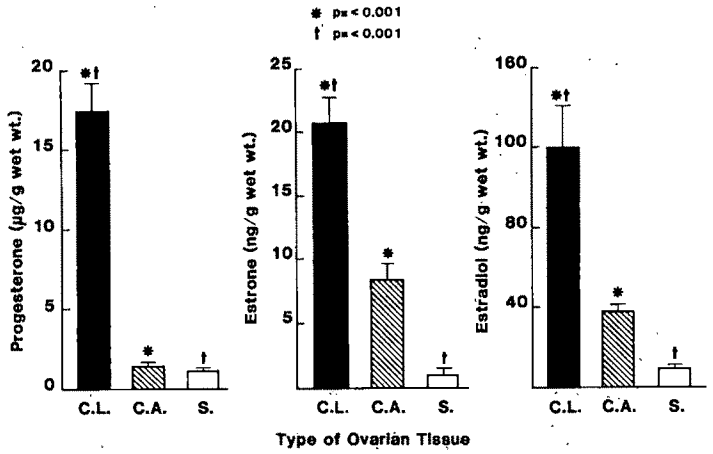


Fig. 4. Mean ± SE concentrations of progesterone, estrone, and estradiol in human corpora lutea *(C.L.)*, corpora albicantia *(C.A.)*, and ovarian stroma *(S)*. The concentrations of these steroids were significantly higher in corpora lutea than in corpora albicantia or ovarian stroma (p = <0.001).

were analyzed for correlation by means of linear regression; p values >0.05 were considered not significant.

Results

Serial dilutions of the corpus luteum extracts produced displacement curves parallel to that of oxytocin in the oxytocin radioimmunoassay. Reverse-phase hydrochloric acid of standard oxytocin (Sandoz) gave a single peak with a retention time of 14.06 minutes for oxytocin under our experimental conditions. A similar corresponding peak with a retention time of 14.06 minutes was also seen in the corpus luteum extract (Fig. 1).

The mean ± SE concentrations of oxytocin in corpora lutea (17.3 ± 2.8 ng/gm wet weight) was significantly higher than in corpora albicantia (1.6 ± 0.2 ng/gm wet weight; p = <0.01) (Fig. 2). Ovarian stroma had undetectable levels of oxytocin.

The concentrations of oxytocin in the corpus luteum increased significantly from the early luteal phase (days 15 to 20) (14.0 ± 1.8 ng/gm wet weight) to reach a maximum (30.8 ± 0.9 ng/gm wet weight) in the mid-luteal phase (days 21 to 25) followed by a significant decline to 8.0 ± 0.8 ng/gm wet weight in the late luteal phase (days 26 to 30; p = <0.001) (Fig. 3).

Fig. 4 shows the concentrations of progesterone, estrone, and estradiol in corpora lutea, corpora albicantia, and ovarian stroma. The mean ± SE concentrations of these hormones were as follows: (1) progesterone, 17.3 ± 1.8 µg/gm wet weight in corpora lutea, 1.4 ± 0.1 µg/g wet weight in corpora albicantia, 1.1 ± 0.1 µg/gm wet weight in stroma; (2) estrone, 20.8 ± 2.0 ng/gm wet weight in corpora lutea, 8.9 ± 0.8 ng/gm wet weight in corpora albicantia, 2.2 ± 0.9 ng/gm wet weight in stroma; and (3) estradiol, 120.5 ± 20.4 ng/gm wet weight in corpora lutea, 37.8 ± 3.9 ng/gm wet weight in corpora albicantia, 9.2 ± 0.9 ng/gm wet weight in stroma. For all three steroids, the corpora lutea had significantly higher concentrations than corpora albicantia or the stroma (p = <0.001). Corpora albicantia also had significantly more estrone and estradiol than the stroma (p = <0.001).

In the corpus luteum, oxytocin concentrations were significantly correlated with estrone (p = <0.025) and estradiol concentrations (p = < 0.005) but not progesterone concentrations (Fig. 5).

The mean ± SE plasma levels of oxytocin are shown in Fig. 6. The plasma levels of oxytocin in the ovarian vein draining a corpus luteum (n = 8) were significantly higher than in the ovarian vein without a corpus luteum during the follicular (n = 10) and luteal phases (n = 14) (p = <0.001) and in the peripheral blood during both the follicular and luteal phases (p = <0.001). There were neither significant differences between plasma oxytocin levels in the peripheral circulation (follicular phase, n = 13; luteal phase, n = 15) and in the ovarian vein without a corpus luteum (follicular phase, n = 10; luteal phase, n = 14) nor between the follicular and the luteal phases.

Fig. 7 shows that there was a significant correlation between corpus luteum concentrations of oxytocin and the ipsilateral ovarian venous plasma levels of oxytocin (r = 0.73, p = <0.05), estrone (r = 0.83, p = <0.025), progesterone (r = 0.96, p = 0.001), and 17α-hydroxyprogesterone (r = 0.77, p = <0.05). There was no significant correlation between corpus luteum oxytocin concentrations and ipsilateral ovarian vein levels of estradiol.

Comment

The present study reports the largest number of human corpora lutea and corpora albicantia ever determined for oxytocin, and for the first time, ovarian vein oxytocin levels are measured and their relationship to

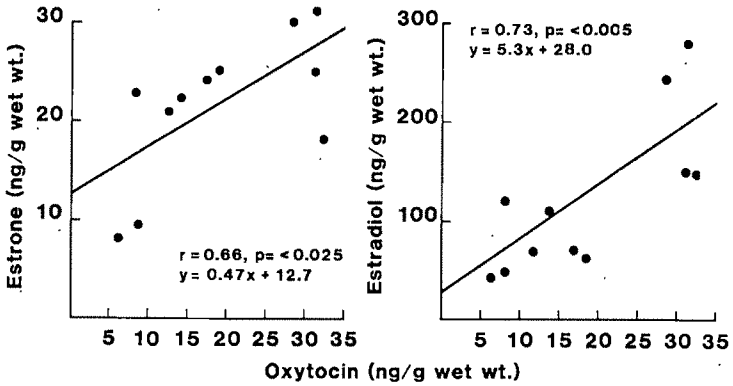


Fig. 5. Correlation between corpus luteum concentrations of oxytocin and estrone and estradiol. There was a significant correlation between oxytocin and estrone and between oxytocin and estradiol concentrations in the corpus luteum.

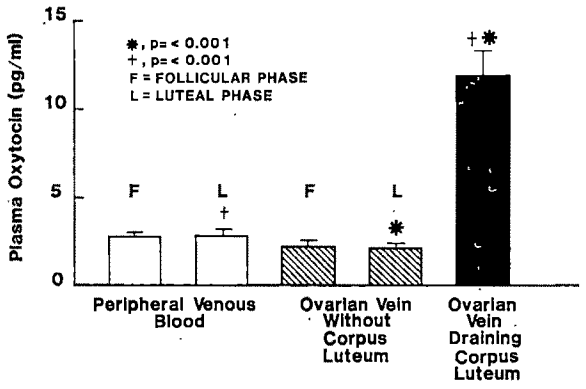


Fig. 6. Mean ± SE of ovarian and peripheral venous plasma oxytocin concentrations. The mean oxytocin concentration in ovarian venous plasma draining a corpus luteum was significantly higher than either ovarian venous plasma not draining a corpus luteum or peripheral venous plasma ($p = <0.001$).

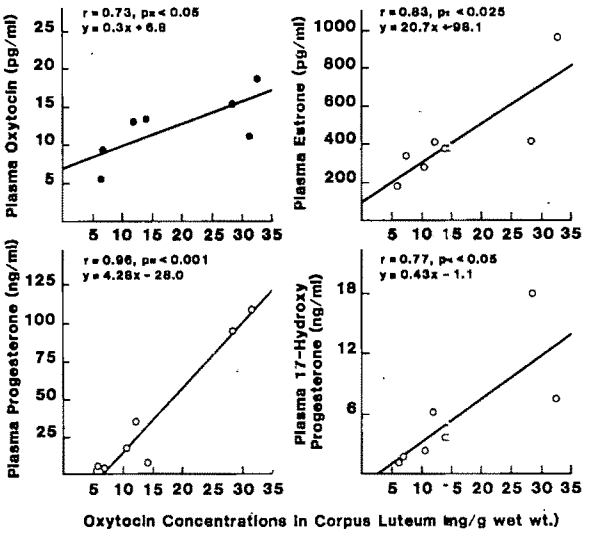


Fig. 7. Correlation between oxytocin concentrations in the corpus luteum and plasma oxytocin, estrone, progesterone, and 17α-hydroxyprogesterone levels in the ipsilateral ovarian vein. There was a significant correlation between corpus luteum concentration of oxytocin and ipsilateral ovarian venous plasma oxytocin, estrone, progesterone, and 17α-hydroxyprogesterone.

ovarian steroid examined. Our findings clearly show that immunoreactive oxytocin is present in human corpus luteum of the menstrual cycle. This peptide-like material is similar to the standard oxytocin as demonstrated by reverse-phase high pressure liquid chromatography (retention time = 14.06 minutes) and by our specific radioimmunoassay, thus confirming the initial observations in both systems.[3,4] The mean oxytocin concentrations of 17.3 ng/gm wet weight in human corpora lutea is similar to that reported earlier in a smaller number of corpora lutea both from our laboratory[4] and by Wathes et al.[3] but are much lower than the concentrations in the corpora lutea of monkeys,[6] cows,[2] or sheep.[2]

Oxytocin is present in the corpus luteum in significantly higher concentrations than in the corpus albicans, but ovarian stroma had undetectable amounts. This suggests that oxytocin is either produced or stored in the corpus luteum. In ruminants, bovine luteal cells and ovine large luteals cells[9] biosynthesize oxytocin in vitro from tritiated leucine. Because oxytocin concentrations in human corpus luteum increased significantly to a maximum level at midluteal phase (days 21 to 25) followed by a significant decline in late luteal phase (days 26 to 30) and a further reduction in the corpus albicans, the active corpus luteum appears to be the source of the oxytocin. This parallelism between the corpus luteum oxytocin concentrations and luteal function vis-à-vis progesterone secretion indicates a probable linkage between corpus luteum oxytocin and luteal steroidogenesis.

This is the first report of oxytocin levels in the ovarian vein which reflects ovarian secretion of oxytocin. In the absence of a corpus luteum the ovarian venous oxytocin

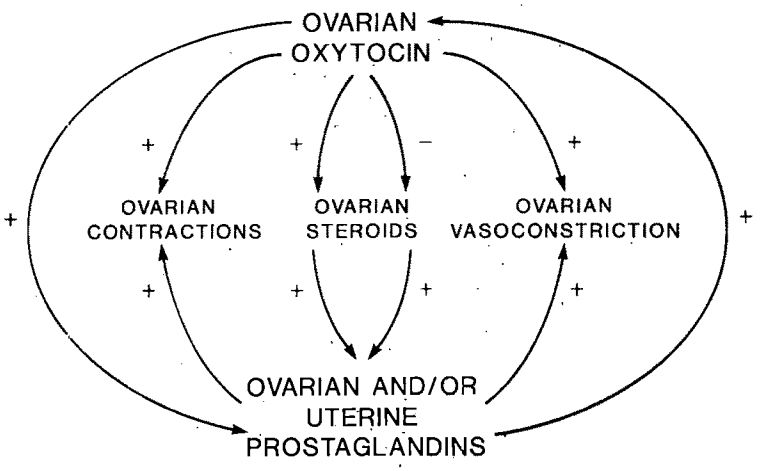


Fig. 8. Hypothesis for the role of oxytocin in human luteal function. The plus sign (+) indicates a stimulatory action while the minus sign (−) indicates an inhibitory effect.

levels are similar to peripheral circulating oxytocin levels, suggesting little or no output of oxytocin from the ovary. When a corpus luteum is present, the ovarian venous effluent has oxytocin levels five times higher than in the contralateral ovarian venous drainage or in the peripheral circulation. This further points to the corpus luteum as the source of oxytocin that is secreted into the ovarian circulation. In the sheep, ovarian venous blood draining the corpus luteum had higher oxytocin levels than jugular venous blood in response to the prostaglandin analog, indicating ovarian secretion of oxytocin.[15]

When corpus luteum oxytocin concentrations are compared to estrone, estradiol, and progesterone concentrations in the same corpus luteum, there is a significant correlation between oxytocin and estrone and estradiol, which suggests that intraluteal oxytocin may directly modulate production and/or secretion of luteal tissue estrone and estradiol levels. The highly significant positive correlation between intraluteal oxytocin concentrations and the ipsilateral venous plasma estrone, progesterone, and 17α-hydroxyprogesterone reflects an in vivo association between luteal tissue oxytocin and its effect on luteal release of its steroid hormones. These findings combined with the parallel rise and decline in corpus luteum oxytocin in mid and late luteal phase vis-à-vis luteal secretion of and circulating levels of progesterone strongly favor a regulatory role of ovarian oxytocin on luteal steroidogenesis and steroid hormone secretion. In this respect, oxytocin was found to be inhibitory on in vitro biosynthesis of progesterone by human corpus luteum cells, but the dose of oxytocin employed was very high.[7] In some nonprimate species,[2] several lines of investigation including oxytocin administration during the luteal phase as well as active and passive immunizations produced variable effects, some of which suggest that oxytocin is a luteolysin.[2] In subhuman primates, direct intraluteal administration of oxytocin shortened the luteal phase and decreased circulating levels.[16] No such direct evidence is available in women, but the results of the present study suggest that luteal tissue oxytocin regulates luteal tissue steroid levels and their release.

Although the precise mechanisms and effect of oxytocin on human luteal function have not been elucidated, we hypothesize that oxytocin may have a permissive luteotropic effect in low tissue concentrations (as in the early luteal phase) and a luteolytic effect (as in the midluteal phase, days 21 to 25) in high tissue concentrations. The luteolytic effect of oxytocin may be mediated through (1) a direct inhibition on luteal steroidogenesis, (2) stimulation of endometrial and/or ovarian prostaglandin, (3) ovarian vasoconstrictive effect, (4) ovarian contractility, or a combination of all these effects (Fig. 8). Further studies are necessary to define the role of oxytocin in human luteal function.

We thank Josie Ramos for excellent assistance in this study.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

DR. CHARLES B. HAMMOND, Durham, North Carolina. The authors are to be congratulated for an excellent paper that investigates human ovarian oxytocin, addresses its source, and attempts to begin to define its relationship to steroid hormone production from the ovary.

It has become increasingly apparent that control of the function of the corpus luteum involves very complex interactions and effects among glycoprotein, polypeptide, and steroid hormones. Luteotropic and luteolytic effects of luteinizing hormone, prolactin, estrogens, progesterone, gonadotropin-releasing hormone, and growth factors have been demonstrated in vivo and in vitro.[1] With the use of the classification system of Sporn and Todaro[2] there is evidence that these luteotropic and luteolytic effects may be achieved by endocrine, paracrine, and autocrine secretion. Unfortunately, a comprehensive description of such effects is hampered by species variability and menstrual cycle phase specificity.

The authors have presented convincing data which support the conclusion that oxytocin is present and is probably produced in the human corpus luteum. I congratulate the authors on their experimental design and their rigorous analytical techniques.

My only concern about this paper is that the authors have made a very significant, inductive leap from their analysis of their data to a model of corpus luteum function that does not seem warranted. Much of the discussion is an attempt to provide a relationship (directly or indirectly) of oxytocin to corpus luteum function. This does not seem feasible with the corre ative analyses used. Correlations do not establish cause The authors have little factual information to suggest that "luteal tissue oxytocin regulates luteal tissue steroid levels and their release." Although they have demonstrated an association between intraluteal oxytocin, estrone, estradiol, and progesterone concentrations, I do not think they have presented sufficient data to support an effect of oxytocin on modulation of luteal steroidogenesis and secretion.

I have several questions for the authors:

1. Oxytocin receptors have been identified in myometrium[3] and in smooth muscle cells of the fallopian tube.[4] Is there evidence that such specific oxytocin receptors exist in the cells of the corpus luteum?

2. What data exist to suggest that ovarian oxytocin secretion is modulated by gonadotropins or gonadal steroids?

3. There remains the issue of the re ationship between oxytocin and prostaglandins. This area clearly needs further study. Some questions to be answered are the following: Does oxytocin secretion result in changes in luteal prostaglandins? What are the comparative "luteolytic" effects of prostaglandin $F_{2\alpha}$ and oxytocin in vitro on human luteal cells? What are luteal concentrations of prostaglandin $F_{2\alpha}$ and oxytocin in the situation of early pregnancy, and do they change similarly or in different fashion? Could oxytocin and prostaglandins influence each other's production? Are both oxytocin and prostaglandins luteolytic, or is one acting via modulation of the other?

Again, I have enjoyed this paper and congratulate the authors. As so usually happens, good research often asks more questions than it answers.

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DR. JOHN P. GUSDON, JR., Winston-Salem, North Carolina. I wonder if this might be a function of the presence of receptors for oxytocin in the corpus luteum?

My other question is, would it be possible, Dr. Dawood, to culture the corpus luteum and simply add a radioactive precursor aminoacid and see whether it is present in the subsequently isolated oxytocin?

DR. FRITZ K. BELLER, Munster, West Germany. In the present patient is there an oxytocinase present in

blood? Since there is an oxytocinase activity available in the blood, is there a local amino peptidase known to be an inhibitor of the corpus luteum?

DR. DAWOOD (Closing). In response to the questions by Dr. Hammond and Dr. Gusdon regarding whether oxytocin receptors are present in the corpus luteum, there are no published data on whether oxytocin receptor is present in human corpus luteum or in the corpus luteum of the cow or the sheep.

We are currently doing some collaborative work that will elucidate this.

In response to Dr. Gusdon's second question, which was whether cultured corpus luteum incubated with radioactive aminoacids will produce radioactively labeled oxytocin, we have carried out preliminary experiments on three corpora lutea obtained on days 21, 23, and 25 with use of radioactively labeled cysteine. Based on our preliminary results, we are able to show that there is bioconversion of radioactive cysteine and its incorporation into a peptide that is identical to oxytocin on reverse-phase high pressure liquid chromatography.

As a follow-up to Dr. Hammond's discussion, we can further show the presence of oxytocin in the human corpus luteum by immunocytochemical localization. We believe that this is the first demonstration of immunocytochemical evidence for oxytocin in the large luteal cells of the human corpus luteum other than previous demonstrations in bovine and ovine corpora lutea.[1, 2] In our laboratory we have just been able to demonstrate in a human corpus luteum of day 21 of the menstrual cycle, dense immunostaining of immunoreactive oxytocin in the luteal cells with a specific oxytocin antiserum by means of the peroxidase-antiperoxidase technique. The immunostained granules are mainly in the cytoplasm of the lutea cells.

Our lead to the role of oxytocin in steroidogenesis was stimulated by the fact that we have found the presence of oxytocin in many human tissues that produce steroids, namely, in the adult adrenal gland, in the fetal adrenal gland, in the testes, and in the ovaries of several species.

This has also been shown in other species by a number of laboratories in Europe. Thus there appears to be some association between steroidogenesis and oxytocin.

Whether oxytocin and steroids regulate one another, that is, oxytocin regulating steroids and steroids in turn regulating oxytocin, remains to be further clarified.

We agree with Dr. Hammond that association does not imply invariably a cause-and-effect relationship, but it implies some kind of a relationship that needs to be further elucidated.

However, based on in vitro studies on human corpora lutea,[3] it has been shown that with use of large doses of oxytocin, as much as 400 ng, secretion and production of progesterone can be significantly inhibited. Therefore there appears to be some direct evidence, at least in vitro, that oxytocin may play a role in the control of human corpus luteum. Our present study is an early attempt to show whether there is such a relationship in vivo.

Is there any effect of oxytocin on prostaglandin? Yes, we believe there is an effect of oxytocin on prostaglandin. This has been shown in many other models but not on the corpus luteum. Perhaps the same mechanism applies in the corpus luteum, but it remains to be shown by direct experimental evidence.

Is there a relationship between oxytocin and prostaglandin in the luteal cells?

Previous studies on measurement of prostaglandin in the luteal cells indicate that the luteal cells have a much lower concentration of prostaglandin $F_{2\alpha}$ than the ovarian stroma. Therefore a relationship may exist between ovarian stroma prostaglandin and luteal oxytocin which is consistent with and invokes the paracrine regulation of the corpus luteum.

Finally, is there a relationship between oxytocin and prostaglandin in the corpus luteum of early human pregnancy?

We have no information about this in early pregnancy but have examined five corpora lutea obtained at elective cesarean section at term, and the amount of oxytocin present in such corpora lutea is low. They are in the range of the oxytocin concentrations found in corpora albicantia. Thus it appears that the concentration and production of oxytocin in such corpora lutea during pregnancy is turned off or inhibited.

In response to Dr. Beller's question whether there is oxytocinase in the blood, yes, it is present in human blood even during the menstrual cycle.

Is there an inhibitor of oxytocin in the corpus luteum? I am not aware of any direct measurements or evidence showing the presence of such an inhibitor, if there is one.

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Midforceps deliveries: Long-term outcome of infants

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Infants delivered by midforceps between 1976 and 1982 were evaluated for evidence of developmental delay or neurological deficit by chart review. The 110 infants who were followed for at least 2 years were compared to a matched group of infants delivered by cesarean section. The cesarean section group was matched for the immediate indication for delivery (dystocia or fetal distress), birth weight, gestational age, sex, and race. There was no significant difference in abnormal outcomes in the two groups. (Am J Obstet Gynecol 1986;154:764-8.)

Key words: Midforceps, infants

Today management of labor is largely directed toward minimizing fetal neurological morbidity and traumatic injury. Electronic fetal monitoring is routinely used in patients at risk for fetal distress and cesarean sections have replaced vaginal delivery as the more frequent birth route when a difficult delivery is anticipated. Potentially traumatic manipulations such as podalic versions have been abandoned. In most cases even the vaginal breech birth has been replaced by cesarean delivery.

The question of the use of midforceps as an acceptable obstetric procedure has been raised. For example, midforceps deliveries have been criticized by some as being potentially traumatic to the fetus[1] and associated with increased perinatal mortality,[2] cerebral palsy,[3] and lower intelligence quotient scores.[4] In contrast, these findings have not been confirmed in other studies.[5-7]

It is recognized that difficult forceps deliveries, particularly from higher stations or with midcavity rotations, may be traumatic to both mother and fetus and are more likely to be associated with abnormal neonatal outcomes when compared to patients with normal labors and spontaneous vaginal births. However, since midforceps births are almost always associated with other risks for morbidity, the impact of the confounding variables such as dystocia and fetal distress is not clear.

In a recent survey of obstetric teaching programs throughout the United States and Canada, all but one of the 73% of respondents stated that the use of midforceps was being taught and that midforceps use varied from <1% to >8%.[8] Thus there appears to be a need for continuing the evaluation of this obstetric technique with respect to morbidity and mortality.

This study represents an evaluation of major neurological morbidity in infants delivered by midforceps in a teaching hospital during a time when midforceps deliveries were generally discouraged. Our aim was to determine whether the use of the forceps resulted in an increase in neurological morbidity when compared to a matched group of patients delivered by cesarean section.

Methods

A retrospective, matched-pair analysis of births at Cleveland Metropolitan General Hospital was conducted on deliveries from July 1, 1976, through December 31, 1982. Data from the antepartum, intrapartum, and neonatal time periods were collected and entered into a database file during the pregnancy and postpartum period. Midforceps deliveries were defined traditionally; the vertex was engaged in the pelvis (station 0 or below) but not resting on the perineum. All forceps rotations from occiput transverse or occiput posterior positions were classified as midforceps deliveries. Labor graphs with timed intrapartum events were generated on each patient at the time of data entry. Patients with fetal deaths before onset of labor, multiple pregnancies, or major congenital anomalies were excluded from the study.

The midforceps procedure was confirmed by chart review. The labors of the midforceps patients and the comparison cesarean birth population were matched for dystocia. Dystocia was defined as protracted active phase, arrest in active phase, or arrest of descent. In addition, the fetal match was for fetal heart rate findings suggesting fetal distress (as derived from the records), birth weight within 200 gm, gestational age within 2 weeks, sex, and race. The maternal charts were reviewed to confirm the characteristics of the midfor-

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Table I. Morbidity of midforceps group (n = 110)

Case No.	*Birth weight (gm)*	*Gestational age (wk)*	*Indication*	*Rotation*	*Age at follow-up (yr)*	*Morbidity**
1	3470	42	Dystocia	–	8	Meconium,* learning disability in speech therapy
2	2490	39	Dystocia	+	4	Group B streptococcus meningitis,* seizure disorder/abnormal electroencephalogram
3	3030	39	Fetal distress	–	2	Cephalohematoma,* neonatal seizures,* language delay
4	3280	39	Fetal distress	–	6	Cephalohematoma,* seizures at age 3, language and motor skills delay
5	4100	40	Fetal distress	+	Neonatal death at 24 hours	Anoxic encephalopathy with subarachnoid hemorrhage at autopsy*

*Asterisks indicate neonatal events.

Table II. Morbidity of cesarean group (n = 110)

Case No.	*Birth weight (gm)*	*Gestational age (wk)*	*Indication*	*Age at follow-up (yr)*	*Morbidity**
1	3110	35	Dystocia	3.4	Severe language delay, expressive skills, intelligence quotient of 58
2	2300	41	Fetal distress	Neonatal death at 14 days	Seizures,* small for gestational age,* subarachnoid hemorrhage*
3	2700	37	Fetal distress	7	Meconium,* moderate speech and language delay
4	4500	40	Dystocia	2.2	Severe speech delay
5	3100	38	Dystocia	2.2	Severe motor delay—not walking at 2 years
6	3120	42	Dystocia	4.2	Meconium,* speech and motor delays
7	2690	39	Fetal distress	2.1	Meconium,* severe language delay
8	2880	39	Dystocia	3.8	Meconium,* motor and mental delay, spastic quadraplegia, seizures at 1.5 yr, death at age 4

*Asterisks indicate neonatal events.

ceps deliveries and to determine the immediate indication for delivery. The infant charts were reviewed to determine whether there were any clinical indications of physical or neurological abnormalities. Mothers of infants who were not followed in the hospital clinics were contacted and interviewed, and their medical histories were reviewed with their managing pediatricians. By study definition all infants had a follow-up evaluation for a minimum of 2 years unless death occurred earlier. Abnormal neurological and developmental outcomes were compared between the two groups with use of the χ^2 statistic.

Results

Midforceps deliveries were performed in 176 deliveries for an incidence of 0.8% (176 of 21,414 births). During the same period primary cesareans were performed in 9.3% of patients. Of the 176 infants delivered with use of midforceps, 110 infant charts were located for follow-up with information for a minimum of 2 years. Nearly all patients were monitored with use of electronic fetal monitoring. Abnormal outcomes were noted in five cases with use of midforceps (5%), as shown in Table I. Abnormal outcomes were observed in eight cesarean section cases (7%) as shown in Table II. One neonatal death was noted in each group. There was an additional death at the age of 2 months from sudden infant death syndrome in the cesarean group. Within each group the primary indications for delivery were evenly divided between dystocia and fetal distress. There were no significant differences in the incidence of clinically apparent abnormal outcomes between infants delivered by midforceps and infants delivered by cesarean section in this study.

Comment

As noted earlier, obstetric practices have certainly changed, and these changes include a decrease in the use of midforceps. Today midforceps applications are probably performed at lower stations than in previous years. However, this fact is more difficult to confirm. As noted earlier, the use of midforceps is still being taught in nearly all training programs.[8] The debate over the use of midforceps centers around their potentially traumatic effects with lasting neurological or intellectual sequelae to the infant.

In the past Eastman et al.[3] and Fuldner[9] demonstrated an association between midforceps delivery and cerebral palsy. Others, however, did not find this association.[10] Friedman et al.,[4] reporting on data from the Collaborative Perinatal Project, documented lower intelligence quotient scores in infants following midfor-

ceps deliveries when compared with spontaneous deliveries matched for labor abnormality. These lower intelligence quotient scores were noted with both protractions and arrests of the active phase and second stages of labor irrespective of delivery route when compared with normal controls (that is, patients with no labor abnormalities and spontaneous vaginal delivery). No comment was made of the intelligence quotient scores in patients with similar labor abnormalities delivered by cesarean section. Other investigators,[11] even those analyzing data from the same Collaborative Perinatal Project,[12, 13] showed no evidence of lowered intelligence quotient scores in midforceps patients.

This study presents clinical follow-up data for at least 2 years on each patient delivered by midforceps compared to a similar group of patients matched for sex, age, weight, race, dystocia, and fetal distress but delivered by cesarean section. Cesarean birth patients were the group selected for comparison, since it was the only alternative for prompt delivery. Vacuum extraction was not performed during this study period. Our data do not indicate that infants born following use of midforceps fared differently than cesarean patients when clinical evidence of neurological deficit was the criterion and when the reason for choice of birth route was controlled.

In these patients neurological abnormalities included delays in language, delays in reaching developmental milestones, seizures, or mental retardation. One neonatal death was noted in each group. Both fetuses showed evidence of hypoxic injury and intrapartum fetal distress. The infant dying of sudden death infant death syndrome is also noted. No implication regarding a causal relationship is made.

A retrospective study such as this has several potential weaknesses. The clinical follow-up of the infants could not be standardized. Care was rendered in many settings and at different ages. Similarly, abnormal outcome measures were based on the clinical evaluations of many different observers. Intelligence quotients were not determined in many children. We anticipate in most circumstances that major neurological sequelae, with clinical impact on development and performance, would be evident clinically.

We cannot address the question of lower intelligence quotient scores in patients delivered by midforceps. However, we note that the control group in Friedman's study consisted of patients matched for dystocia rather than fetal distress, a condition commonly associated with midforceps deliveries. Similarly, comparison was not made with patients delivered by cesarean when matched for labor abnormality.

It would appear that major concern for abnormal outcome would relate to outcome measures of clinical significance including learning disabilities, speech delays, cerebral palsy, and spastic quadraplegia. These are conditions linked with handicap in normal function.

One cannot conclude from this study that midforceps are safe. The operative procedure was infrequently used (0.8%) and generally from the +2 station or lower. One might interpret this as selection of only the relatively "easy" midforceps candidates. This attitude may well have been a factor, since our staff members no longer feel comfortable performing midforceps deliveries and tend only to perform those that they anticipate can be accomplished without difficulty. It is entirely possible that, if midforceps were done more frequently, more difficult midforceps procedures would be performed with higher risk to the infant. With the reservations we have described, it does appear that selected midforceps deliveries may be performed with no greater risk of abnormal outcome than that associated with cesarean section.

We do not plan to encourage more frequent use of midforceps in our residents' education program nor do we advocate their use electively for "training" purposes. We request that a faculty member be present for midforceps deliveries when possible. When they are used cautiously, we do feel that midforceps deliveries have a place in modern obstetric care.

Finally, we are not advocating a return to difficult midforceps procedures. When the alternative to cesarean is midforceps delivery, the use of the forceps remains a judgment decision of the obstetrician at the bedside. In the absence of evidence of fetal stress, however, continued observation, as described by Cohen,[14] may be the wisest choice.

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Discussion

DR. E. J. QUILLIGAN, Sacramento, California. The authors are to be congratulated for they wisely have chosen to compare patients delivered with use of midforceps with those delivered by cesarean section for similar indications (dystocia and fetal distress). This is the only study that I know of which is matched in this manner. They have further matched the patients delivered by forceps and cesarean section according to gestational age, birth weight, race, sex, and type of labor dystocia. The only important variable that they have failed to match is the socioeconomic grouping. This may have been an automatic match in their population and is most important when examining less severe neurological impairment. The single bias that cannot be matched for is whether those patients delivered by cesarean section would have been difficult forceps deliveries; had the easy forceps deliveries alone been delivered by cesarean section, the neurological damage would have been less. The only way to answer this question properly is with a prospective randomized study. This study provides a firm rationale for conducting such a prospective study.

The results of their study may gladden the hearts of those who would like to decrease the cesarean section rate and those who feel that residents can be trained to do midforceps deliveries safely. Will either objective be accomplished with use of the criteria they must have used for a midforceps delivery? The cesarean section rate could be reduced by 0.8%, which is not a huge decrease in light of the marked increase that has occurred during the last 20 years (from 7% to 20%). However, any decrease is important, and it is only by chipping away at the high cesarean section rate that it will be reduced.

The resident training question is more difficult to answer because the resident must learn two things: (1) the judgment as to which patient is properly delivered with use of midforceps and (2) the technique necessary to do a safe midforceps delivery. If my calculations are correct, the Cleveland Metropolitan Hospital Obstetrics Service has 27 to 29 midforceps deliveries per year. Since they have five residents per year, each resident would be responsible for five to six midforceps deliveries during their training. Is this enough? A quick look at the numbers would yield a "no" answer, but a different analysis might say "yes." I think this is an important question because it applies to other areas of obstetrics and gynecologic residency training in which small numbers of patients may be available, such as vaginal delivery of a breech and some surgical procedures such as vaginal hysterectomy. There is no magic number that will assure that a resident has proficiency in either clinical judgment or surgical technique. The staff must judge each resident on an individual basis, which leads to a question concerning Dr. Rosen's data. How frequently was a staff member present when the resident did a midforceps delivery? In a previous article[1] with use of the same subset of patients, 15% of the patients were delivered by the staff. Are these the only patients who had a staff member present at delivery? Assuming that a staff member is present at each procedure and is evaluating the residents, how can our review boards be certain that the evaluation is a proper one? One way is to look at mortality and morbidity, comparing trends in both at the same institution and comparing one institution against some norms of other institutions with similar patient populations. What I am saying is that perhaps our residency review committees should look at criteria other than the numbers of cases per resident to evaluate training.

Referring again to this article, I was surprised at the high incidence (5% to 7%) of severe neurological abnormalities at 2 years of age in these patients, particularly the group delivered for dystocia. With use of the previous article's total of 61 patients with fetal heart rate abnormalities and the assumption that the remaining 115 patients were delivered for dystocia, this would yield a 3% incidence of neurological abnormalities in this population, a figure 30 times higher than the generally quoted ratio of 1:1000 in all deliveries. The incidence of neurological abnormality when delivery was performed for fetal distress was 5:122 or 40:1000 deliveries. Again, a very high figure, assuming these were primarily fetal heart rate abnormalities found during the second stage of labor. Would Dr. Rosen comment on these aspects of their study?

Finally, I would like to congratulate the authors for the best retrospective study that I have seen on this subject.

DR. CHARLES A. WHITE, New Orleans, Louisiana. As I listened, three questions occurred to me.

One, I was expecting to hear some information about the Apgar scores of the infants, and wonder if you would comment.

Second, I noticed in your two groups that there appeared to be only one infant in each group with a birth weight in excess of 4 kg. Do you have a particular form of management, perhpas a more liberal attitude toward cesarean section, in those infants who are recognized during labor or before labor to be in a macrosomic weight range?

Third, as obstetricians we must be concerned not only about the antenatal and the intrapartum events but also about the neonatal events. If you continue this study,

or if you were to begin a new study design, would you see any advantage in routine study of cord blood information, particularly pH and blood gases, to help in prediction of outcome in these newborn infants?

Dr. Edgar L. Makowski, Denver, Colorado. Do you have any data on umbilical cord gas studies comparing the two groups? Such results are critical in a study like this.

Dr. A. Brian Little, Montreal, Quebec, Canada. I have two questions to ask. I have been impressed over the years by Mortimer Rosen's very conservative practice of obstetrics, and the obvious question which has already been alluded to is, how does his population, not only his population of patients but his population of faculty, influence these results in view of his strong control of the obstetrics that is practiced at his institution?

The reason I say this is because my colleagues, Robert Usher and Mark Boyd, have recently reviewed our midforceps results; in the presence of our increasing cesarean section rate over a long period of time, there has also been an increase in the midforceps rate, and these are all performed with a faculty member being present.

The second question I would like to ask relates again to my two colleagues who have brought up a concept that I immediately rejected called "failed forceps." I do not know what failed forceps are. I have never had a case, and I know my learned colleagues in this room have never had one.

However, there appears to be an incidence of failed forceps in our institution which is on the increase. I am not sure what it means. Do you?

As a corollary to that, we have an increased number of complications following cesarean sections late in labor. Do you have a similarly increased incidence of complications?

Dr. James A. Low, Kingston, Ontario, Canada. I would like to pose a question in that we are now at a point where we must have morbidity measures to meaningfully assess the implication of different obstetric events.

One of the problems identified in this study is measuring morbidity at 2, 4, 6, and 8 years of age. I would like to ask the general question: Would they agree that it is time that we standardize our outcome measures and the methods used and begin to work collaboratively with developmental psychologists and pediatric neurologists in regard to these measures of morbidity.

Dr. Dierker, (Closing). First, the socioeconomic status of the patients was not coded prospectively in the computer at the time. When we evaluated the relative incidence of private versus staff cases, we found that 15% of the midforceps patients were private and 20% of the cesarean section patients were private, which is not dissimilar but they were not matched.

Another question regarding the staff being present as noted in our first paper, the most senior person evident by review of the delivery note was mentioned as the deliverer, so if a faculty person was present in the room, we counted him as the senior person being present; based on these criteria, as best as we could determine from our first paper, they were fairly equally distributed between second, third, and fourth year residents and faculty.

Dr. Quilligan noted the neurological abnormalities that seemed to be a fairly high incidence noted in both dystocia and distress groups. I certainly agree that it is higher than the national average for normal deliveries. However, this is a select population, since 42% of them had dystocia and 42% of them also had fetal distress in both groups.

Regarding the selected population, the population of our hospital is about 80% staff and 20% private.

Regarding Dr. White's comments, the incidence of low Apgar scores was the same in the cesarean section group and in the midforceps group. We had a 1-minute Apgar depression of about 32% and 6% for the 5-minute Apgar score.

We did not have cord blood gas data on all the patients. We did have the indication of asphyxia noted in the neonatal record defined by acidosis and apparent depression, and they were the same, 14% in each group. They were not matched. It just happened that way.

Regarding the apparently few cases of large for gestational-age infants, when we reviewed our initial series of 176, we found that the incidence of large for gestational-age infants was no higher in our midforceps group than in our general population, a finding that was a surprise to me, but it was the same as the normal population.

Dr. Makowski wondered about the umbilical cord gas data, but we did not have umbilical cord gas studies. We did have scalp sampling on a number of patients, but as I mentioned, there was a 14% incidence of asphyxia clinically and documented by blood gas studies in the newborn infant at the time of admission to the neonatal intensive care unit.

Regarding Dr. Little's comments on failed forceps, we rely on our computer search to determine whether we had any failed forceps. I could only locate 10 in our review of that same time period, which leads me to believe that they are not being coded as frequently as they probably occur.

He also had a question about the population—how we keep the residents from choosing cesarean section.

I think that our general policy is to encourage vaginal delivery. We use fetal scalp sampling liberally with fetal monitoring in virtually all of our cases of dystocia and allow the patient to continue vaginally as long as she is making progress until vaginal delivery.

Regarding the comments by Dr. Low, I agree that standardized follow-up is essential if we are going to compare outcome measures. It is our sincere hope that we will have the opportunity and the economic support to follow these infants prospectively when they reach appropriate age with suitable testing in the future.

The relationship between antepartum fetal heart rate, intrapartum fetal heart rate, and fetal acid-base status

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The relationships between antepartum baseline fetal heart rate, baseline variability, accelerations, decelerations, and fetal movement and intrapartum fetal heart rate, fetal acid-base assessment at delivery, and Apgar scores 1 and 5 minutes after delivery were studied in 290 mature pregnancies. There was a significant relationship between antepartum and intrapartum baseline fetal heart rate and baseline variability. There was no relationship between antepartum and intrapartum accelerations or decelerations. There was no correlation between antepartum fetal heart rate or fetal movement and fetal acid-base status at delivery or Apgar scores 1 and 5 minutes after delivery. There was a trend for decreased accelerations and decreased fetal movements to be associated with a low Apgar score 1 minute after delivery. (AM J OBSTET GYNECOL 1986;154:769-76.)

Key words: Fetal heart rate, fetal hypoxia

Antepartum fetal heart rate monitoring has become a widely accepted method of surveillance of the fetus at risk for uteroplacental insufficiency.[1, 2] However, reports of the role of the nonstress test have highlighted a number of problems.

Standardization of the test and its interpretation have not been achieved. Many investigators recommend a fetal heart recording of at least 40 minutes with the expectation that a healthy fetus should demonstrate two to five accelerations of a minimum of 15 bpm and 15 seconds.[2] Studies of the fetal state in normal pregnancies have demonstrated that the interval between successive accelerations may be 37 minutes and the interval between fetal movements as long as 75 minutes.[3] This has led to the recommendation that the recording of fetal heart rates should be extended to 80 to 120 minutes before being classified as nonreactive. Fetal heart rate accelerations are the most frequently used indicator of fetal reactivity. However, some authors continue to include baseline fetal heart rate, baseline variability, and decelerations in the identification of the compromised fetus.[4-7] These observations have been used to provide a composite score or describe a "terminal" record.

Studies of the predictive value of the nonstress test have used differing measures of outcome including fetal growth retardation, fetal distress during labor, fetal acidosis at delivery, low Apgar score, and fetal and neonatal deaths, often without adjustment for the confounding effect of gestational age. Nevertheless, a number of general conclusions have emerged from these studies.

The nonstress test has been criticized because of the low positive predictive value of a nonreactive test. False positive nonreactive fetal stress tests are usually reported to be in excess of 50%.[5, 8] The number of false positives can be reduced by extending the duration of the nonstress test to 80 minutes[9] or increasing the criteria required to define a nonreactive test.[5] However, these adjustments will decrease the sensitivity of the test.

It is generally agreed that a reactive nonstress test has a good negative predictive value providing reassurance about fetal well-being and a low incidence of fetal death.[2, 6, 10, 11] The incidence of false negative reactive nonstress tests in relation to a number of mortality and morbidity outcome measures has varied from 1.5% to 7%.[5, 8, 9, 12] However, there is some concern. The majority of tests are reactive (>90%). Thus the low false negative percentage represents a significant number of cases. Prospective studies of the nonstress test have in general had few fetal deaths. Yet fetal deaths with a reactive nonstress test are reported in most studies,[10] and a number were noted in a multi-institutional study reported by Freeman et al.[1]

The aim of this test is to detect those pregnancies with uteroplacental insufficiency and prevent fetal death. This implies that antepartum fetal heart rate can predict fetal hypoxia, which in turn may lead to morbidity or death during the subsequent perinatal period.

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Table I. Antepartum maternal and obstetric risk factors in the 290 pregnancies

	No.
High-risk pregnancies	212
Past obstetric complications	10
Prior stillbirth	8
Prior neonatal death	2
Maternal medical complications	92
Hypertension	70
Renal disease	3
Diabetes	11
Other	8
Obstetric complications	43
Toxemia	39
Antepartum hemorrhage	3
Other	1
Gestational complications	113
Postterm	94
Multiple pregnancy	7
Growth retardation	11
Other	1
Low-risk pregnancies	78

The objective of this study was to examine this question in high-risk and low-risk pregnancies at term. The antepartum fetal heart rate characteristics obtained within 24 hours of delivery were studied in regard to their association with intrapartum fetal heart rate, intrapartum fetal hypoxia with metabolic acidosis at delivery, and low Apgar scores 1 and 5 minutes after delivery.

Methods

The study population consisted of 290 obstetric patients with a mature fetus who were admitted to the hospital for induction of labor. Two hundred twelve obstetric patients were classified as having a high-risk pregnancy because they exhibited one or more of the maternal or obstetric antepartum risk factors outlined in Table I. The remaining 78 pregnancies were classified as low-risk pregnancies.

Complications of labor included meconium in the amniotic fluid (53 patients [18%]) and abnormal uterine action or cephalopelvic disproportion (34 patients [12%]). There were 234 vaginal deliveries and 53 cesarean sections.

A 40-minute recording of antepartum fetal heart rate and fetal movement was obtained within 18 hours of the induction of labor. After induction of labor, a continuous recording of intrapartum fetal heart rate was obtained in those patients managed in the intrapartum intensive care unit. An acid-base assessment of umbilical vein and artery blood and an Apgar score at 1 and 5 minutes were obtained at delivery.

Antepartum fetal heart rate was recorded with Hewlett-Packard external fetal monitor model No. 8040A (Hewlett-Packard Co., Palo Alto, California), the patient having refrained from smoking and nourishment for at least 1 hour before the test. Forty minutes of satisfactory recording was obtained by a research assistant while the mother recorded fetal activity. Each record was scored by two research assistants. Any discrepancies were reviewed with one author (J. A. L.). The fetal heart rate was scored according to the following criteria.

Table II. Composite score of fetal heart rate and fetal movement

	0	*1*	*2*
Baseline rate	>160	141-160	≤140
Baseline variability	≤5 (×2)	≤5 (×1)	≥6
Accelerations	0	1	≥1
Decelerations	>1	1	0
Movements	0	1	>1

Baseline fetal heart rate was the rate in the intervals between uterine contractions or episodes of marked fetal activity. Fetal heart rate was scored for the first and second 20 minutes and averaged. An abnormal finding was a fetal heart rate >140 bpm.

Baseline variability represents the fluctuations of the baseline fetal heart rate. Baseline variability for the two 20-minute periods was classified according to the amplitude of the fluctuations as normal (≥5 bpm) or decreased (≤5 bpm). An abnormal finding was decreased baseline variability for one or two 20-minute periods.

Fetal heart rate accelerations are short-term increases of fetal heart rate of at least 15 bpm above baseline. The number of accelerations in 40 minutes was noted. An abnormal finding was a record with less than two accelerations in 40 minutes.

Fetal heart rate decelerations are transitory declines of the fetal heart rate by at least 15 bpm below baseline. The number of decelerations in 40 minutes was noted. An abnormal finding was a record with one or more decelerations in 40 minutes.

Fetal movements were indicated on the record by an event marker operated by the mother. The number of fetal movements in the 40 minutes recorded was noted. An abnormal finding was a record with fewer than two fetal movements in 40 minutes.

A composite score of fetal heart rate and fetal movement was calculated according to the criteria in Table II.

The continuous recording of intrapartum fetal heart rate was obtained with a scalp electrode applied at the time of induction of labor in 115 patients. For this study, each 20-minute period of the first hour and the last 3 hours of the fetal heart rate record was scored by a research assistant without awareness of the clinical characteristics, antepartum fetal heart rate, or laboratory data.

Table III. Means, SDs, range, and incidence of observations of abnormal fetal heart rate and fetal movement in the 40-minute antepartum record

	Mean	*SD*	*Range*	*Abnormal results*	
				No.	*%*
Baseline rate	137.7	8.8	112-172	72	25
Baseline variability	6.1	1.0	3-15	66	23
Total accelerations	9.2	5.4	0-27	18	6
Total decelerations	1.1	2.1	0-17	133	47
Fetal movement	18.2	20.5	0-125	19	7
Composite score	8.6	1.3	4-10	50	17*
				9	3†

*Score ≤7.
‡Score ≤5.

Table IV. Correlations between observations of corresponding antepartum and intrapartum fetal heart rate; correlation for deceleration was between the number of antepartum decelerations and the percent of intrapartum decelerations

Antepartum fetal heart rate	*Intrapartum fetal heart rate*			
	First hour		*Last 3 hours*	
	R	*p*	*R*	*p*
Baseline rate	0.38	0.0001	0.31	0.001
Baseline variability	0.34	0.0002	0.26	0.01
Accelerations	0.11	NS	0.10	NS
Decelerations	0.08	NS	0.05	NS

The criteria for baseline fetal heart rate, baseline variability, and fetal heart rate accelerations and decelerations were similar to those used in the antepartum period. Fetal heart rate decelerations were classified as variable or variable late decelerations.

Variable decelerations vary markedly in shape from contraction to contraction. The departure and return of fetal heart rate is abrupt and the lag time is variable.

Variable late decelerations are characterized by an onset late in the contraction phase, and the maximum point of the deceleration occurs more than 20 seconds after the acme of the contraction.

Total decelerations are the sum of variable and variable late decelerations. Total decelerations were classified according to the percent of contractions associated with a deceleration as follows: moderate, 5% to 30% and marked, >30%.

Fetal blood gas and acid-base assessments were performed on umbilical vein and artery blood at delivery. Measurements of pH were made with a microelectrode pH meter (Astrup AME-1). Oxygen tension and carbon dioxide tension were measured with an IL-113 system. Buffer base was calculated from the Singer Hastings nomogram and corrected for the degree of oxygen unsaturation. Evidence of fetal hypoxia was based on the presence of a significant degree of metabolic acidosis in the umbilical artery (umbilical artery buffer base <34 mmol/L).

Apgar scores were obtained at 1 and 5 minutes by an independent observer using a stop watch.

Statistical analysis between antepartum fetal heart rate and intrapartum fetal heart rate and measures at delivery included Student *t* tests and χ^2 analysis. Correlation coefficients were established for each relationship.

Results

The means, SDs, range, and incidence of abnormal results for the antepartum fetal heart rate and fetal movement are presented in Table III. The incidence of abnormal results were: fetal tachycardia (>140 bpm), 25%; decreased baseline variability (≤5 bpm), 23%; accelerations (≤1), 6%; decelerations (≥1), 47%; and fetal movements (≤1), 7%. Abnormal composite scores were defined as either ≤7 (17%) or ≤5 (3%).

The significance of antepartum risk factors in relation to no risk factors, and after term in relation to term pregnancies, with respect to antepartum fetal heart rate and fetal movement was analyzed. Baseline fetal heart rate was slightly lower in high-risk in relation to low-risk pregnancies (136 versus 139 [$p < 0.01$]) and after term in relation to term pregnancies (135 versus

Table V. Association between abnormal antepartum fetal heart rate measures and the corresponding abnormal measures during the first and the last 3 hours of labor; the association for decelerations was analyzed with marked deceleration patterns (>30% contractions with decelerations) during the first and last 3 hours of labor

	Antepartum		*Intrapartum*			
			First hour		*Last 3 hours*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Baseline rate >140 bpm	28	24	11/32	34	16/51	31
Baseline variability <5 bpm	26	23	13/35	37*	17/58	29*
Accelerations <1	6	5	2/17	12	1/8	13
Decelerations >1	52	45	18/29	62†	35/66	53†

*$p < 0.01$.
†$p < 0.05$.

Table VI. Range of umbilical artery buffer base at delivery and Apgar scores 1 and 5 minutes after delivery

Umbilical artery buffer base		*Apgar scores*		
mmol/L	*No.*	*Score*	*1 minute (No.)*	*5 minutes (No.)*
>38	157	7-10	217	232
34-38	87	4-6	57	7
<34	28	0-3	15	0

137 [$p < 0.04$]). Otherwise no significant differences were observed for baseline variability, accelerations, decelerations, or fetal movements.

Relationship between antepartum and intrapartum fetal heart rate. The correlations between the corresponding observations of antepartum and intrapartum fetal heart rate are presented in Table IV. There is a significant correlation between antepartum baseline rate and intrapartum baseline rate in the first and last 3 hours of labor. Similarly, there is a significant correlation between antepartum baseline variability and baseline variability in the first and last 3 hours of labor.

However, there is no correlation between the number of accelerations or decelerations in the antepartum record and the number of accelerations or the percent of decelerations during the first or the last 3 hours of labor.

Variable late or late decelerations were observed in five patients (4.0%) during the first hour of labor and in 27 patients (23%) during the last 3 hours of labor. There was no correlation between the number of decelerations in the antepartum record and the number of late decelerations during the first hour of labor (R 0.03) or the last 3 hours of labor (R 0.01).

The associations between observations of abnormal antepartum fetal heart rate and the corresponding intrapartum fetal heart rate are outlined in Table V.

Baseline tachycardia and decreased baseline variability in labor is more likely to occur in patients with baseline tachycardia and decreased baseline variability in the antepartum period.

There is no evidence that decreased antepartum accelerations are predictive of decreased accelerations during labor. Although there was no correlation between the number of antepartum decelerations and the percent of decelerations during labor, there is a trend for the patients with antepartum decelerations to have a greater incidence of patterns of marked deceleration. No association was found between antepartum baseline tachycardia, decreased variability, decreased accelerations or decelerations, and variable late or late decelerations during the first and last 3 hours of labor.

Relationship of antepartum fetal measures to outcome measures at delivery. The range of umbilical artery buffer base at delivery and the Apgar scores at 1 and 5 minutes are presented in Table VI. The significance of antepartum risk factors and duration of labor to the outcome measures was analyzed. There is a trend to a greater incidence of metabolic acidosis (umbilical artery buffer base <34) in high-risk in relation to low-risk pregnancies (12% versus 5% [$p < 0.07$]) and long labor (>7 hours) in relation to short labor (13% versus 6% [$p < 0.03$]).

The correlation coefficients between each antepar-

Table VII. Correlation coefficients between antepartum fetal heart rate and fetal movement and umbilical artery buffer base and Apgar scores 1 and 5 minutes after delivery

	Umbilical artery buffer base		*1 Minute*		*5 Minutes*	
	R	*p*	*R*	*p*	*R*	*p*
Baseline rate	0.01	NS	−0.03	NS	0.01	NS
Baseline variability	0.05	NS	−0.06	NS	−0.11	NS
Accelerations	0.01	NS	−0.02	NS	−0.05	NS
Decelerations	−0.02	NS	−0.03	NS	0.03	NS
Fetal movement	−0.02	NS	−0.02	NS	0.02	NS
Composite score	0.10	NS	0.08	NS	−0.01	NS

Table VIII. Association between abnormal antepartum fetal heart rate and fetal movement and fetal metabolic acidosis at delivery (umbilical artery buffer base <34 mmol/L) and low Apgar scores 1 minute after delivery

	Umbilical artery buffer base				*Apgar score 1 minute*			
	≥34 mmol/L		*<34 mmol/L*		*≥7*		*<7*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Baseline rate >140 bpm	63	26	6	22	50	23	22	31
Baseline variable <5 bpm	53	22	8	30	49	23	18	25
Accelerations <1	16	7	2	8	11	5	7	10*
Decelerations >1	113	47	14	54	98	46	35	49
Movements <1	14	6	4	15	11	5	8	12*
Composite score <7	44	18	5	18	35	16	15	21

*$p < 0.05$.

tum fetal heart rate measure and the outcome measures are presented in Table VII. There are no significant correlations between antepartum fetal heart rate or fetal movements individually or as a composite score and umbilical artery buffer base at delivery or Apgar scores at 1 and 5 minutes.

The association between abnormal antepartum fetal heart rate and fetal movement and fetal metabolic acidosis and low Apgar scores at delivery is presented in Table VIII. There is no association between abnormal antepartum fetal heart rate or fetal movement and fetal metabolic acidosis. No consistent association was observed between abnormal antepartum fetal heart rate and fetal movement and low Apgar scores at 1 minute. However, there was a trend for decreased accelerations and decreased fetal movement to be associated with a low Apgar score at 1 minute.

An Apgar score less than 7 at 5 minutes was observed in seven newborn infants. There was no association between abnormal antepartum fetal heart rate and fetal movement and low Apgar scores at 5 minutes in this small number of newborns.

Comment

The low-risk patients with no major antepartum risk factors had the same incidence of abnormal antepartum fetal heart rate and fetal movement as the high-risk patients. This is in keeping with previous reports.[11] The incidence of less than two accelerations per 40 minutes was 6%. This closely parallels the frequency of nonreactive nonstress test reported in studies of high-risk pregnancies that have used this criterion alone.[1, 6, 8, 11]

The significant association between antepartum and intrapartum baseline rate and baseline variability suggests continuity of fetal state as expressed by these measures of fetal heart rate.

Intrapartum fetal heart rate, particularly patterns of total decelerations and variable late or late decelerations, are important predictors of intrapartum fetal hypoxia. There is no evidence that observations of antepartum fetal heart rate can predict either marked patterns of total decelerations or variable late or late decelerations during labor.

The best criterion of intrapartum fetal hypoxia is a fetal acid-base assessment with evidence of a significant metabolic acidosis. A number of reports of the significance of the nonreactive nonstress test have used umbilical vein or artery pH as one indicator of fetal distress or outcome morbidity.[4, 5, 9, 12] A recent study of elective sections has reported a trend to a higher incidence of umbilical vein pH <7.26 in infants with a nonreactive nonstress test before delivery.[13] No systematic study of the relationship of antepartum fetal heart rate and fetal acid-base status during labor has been reported.

In the present study, no correlation was observed

between antepartum fetal heart rate or fetal movement and umbilical artery buffer base at delivery. A significant metabolic acidosis (umbilical artery buffer base <34 mmol/L) was observed in 28 (10%) of the newborns at delivery. There was no association between observation of abnormal antepartum fetal heart rate or fetal movement and a significant metabolic acidosis at delivery. This implies that observations of abnormal fetal heart rate or decreased fetal movement cannot predict intrapartum fetal hypoxia within the next 24 hours. This was equally true in patients with a long labor (>7 hours) or a short labor (≤7 hours).

Apgar scores have been one of the outcome measures in a number of the studies of the nonstress test. The incidence of a low Apgar score at 1 minute (<7) has in general been greater after the nonreactive than the reactive nonstress test. The data from three reports[6, 7, 15] indicate that low Apgar scores are at least twice as common after a nonreactive nonstress test. Although there was no correlation between antepartum fetal heart rate or fetal movement and Apgar scores at 1 minute in the present study, there was a trend for decreased accelerations and decreased fetal movements to be associated with a greater incidence of low Apgar scores with a difference in incidence of the order of 2.

Most studies have placed greater emphasis on Apgar scores at 5 minutes as an indicator of fetal depression caused by hypoxia. The number of low Apgar scores at 5 minutes in most studies is usually less than 10. However, there was a trend to a greater incidence of low Apgar scores at 5 minutes in the multi-institutional study reported by Freeman et al.[1] There was no evidence of an association between antepartum fetal heart rate or fetal movement and the small number of low Apgar scores at 5 minutes in this study.

The stated aim of observations of antepartum fetal heart rate is to identify the initial manifestations of uteroplacental insufficiency, presumably expressed first by transient periods of mild or moderate hypoxemia with normal acid-base balance and subsequently by hypoxemia with metabolic acidosis. Studies in the fetal lamb and fetal monkey have provided valuable evidence of the fetal heart rate response to fetal hypoxemia. Baseline fetal heart rate tachycardia after an initial bradycardia has been demonstrated with moderate hypoxemia.[14] Hypoxemia with mild metabolic acidosis in fetal lambs resulted in no change in the number, amplitude, or duration of accelerations.[15] A decrease of accelerations is a late phenomenon associated with severe hypoxemia and acidosis.[16] Fetal heart rate decelerations occur when fetal oxygen tension falls below a critical level.[17] Decelerations in the fetal monkey occur with progressive hypoxemia before the development of metabolic acidosis.[16] There is an increase in the number and amplitude of decelerations in the fetal lamb with moderate hypoxemia with mild metabolic acidosis.[15]

These findings indicate that a moderate degree of hypoxemia is required to cause changes in fetal heart rate. Also baseline fetal heart rate tachycardia and appearance of decelerations rather than the disappearance of accelerations is the earlier sign of fetal compromise. Thus it is not surprising that observations of antepartum fetal heart rate cannot predict fetal hypoxia of uteroplacental insufficiency occurring 12 to 24 hours later with labor but rather reflects the state of the fetus at the time the observations were made.

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Discussion

DR. ROBERT RESNIK, San Diego, California. For several years I have had the opportunity to learn from the careful clinical investigations carried out by Dr. Low and his colleagues from Kingston. His studies of the immediate neonatal, as well as long-term outcomes of growth-retarded infants, and those subjected to birth asphyxia in general have been significant contributions to our understanding of these pathophysiologic states.

Dr. Low has presented data that question the sensitivity of the nonstress test with respect to its ability to predict intrapartum fetal heart rate, baseline variability, and periodic changes, as well as neonatal outcomes reflected by acid-base status at delivery and Apgar score. His data do demonstrate that antepartum fetal heart rate monitoring will accurately predict intrapartum heart rate and variability in labors occurring within 24 hours of testing. However, he found no correlation with heart rate decelerations, acid-base status, or Apgar score. Indeed, among seven newborns with 5-minute scores of less than 7, neither antepartum fetal heart rate nor fetal movement activity was abnormal before labor. In short, the results support his conclusion that the nonstress test may not accurately predict fetal hypoxia in labors occurring within 24 hours of the test.

In all fairness, this may be too much to expect from the nonstress test. The loss of baseline variability and fetal accelerations with fetal movement are later rather than earlier signs of hypoxia. When the test is reactive, its proponents claim only that the fetus is well at that point in time and that 98% of fetuses will survive another week of pregnancy; they also concede a high false positive rate. However, Dr. Low's findings do focus our attention on the continuing debate regarding the relative merits of nonstress and contraction stress testing, as well as the biophysical profile, in their role as antepartum measures of fetal well-being. If one dispassionately evaluates the currently available data, it seems that all three tests are quite satisfactory, when properly selected and used with good clinical judgment. This statement is supported by the findings of Freeman et al.[1] in a multi-institutional assessment of the nonstress test and contraction stress test in high-risk pregnancies. They found the corrected perinatal mortality rate to be seven per 1000 among pregnancies followed with the nonstress test as the primary means of surveillance compared with three and a half per 1000 among those followed by the contraction stress test alone. Although some have concluded that these data show the contraction stress test to be a better predictor than the nonstress test by a factor of 2, it seems to me that a more appropriate conclusion is that both tests result in enviable perinatal mortality rates.

Nevertheless, studies in several animal models, as well as clinical observations, confirm that a late deceleration is an earlier sign of fetal hypoxia and that the loss of fetal heart rate variability and accelerations is a later consequence of ensuing acidosis. Following this line of reasoning, a contraction stress test would logically be an earlier and more sensitive indicator of hypoxia.

At the current time, there is considerable interest in tailoring the antepartum test to the specific fetal disease entity and combining these results with ultrasonic estimates of amnionic fluid volume. Data regarding predictive value of the biophysical profile are also quite promising, and it seems reasonable that eliciting a multitude of fetal responses is superior to observations of a single physiologic paramater. Parenthetically, I should point out that for two decades we obstetricians have searched for a single test that will tell us whether the fetus is healthy. This is unrealistic in as much as no such test exists for the more accessible adult organism.

Why did you use a 40-minute time limit for antepartum monitoring? When one considers the normal sleep and wake cycles of a fetus and their impact on variability, together with the observations of Brown and Patrick,[2] that the predictive accuracy of nonstress testing can be improved with an 80-minute observation, it seems likely that a longer test time would have improved the sensitivity. Second, it seems that a definition of a fetal heart rate greater than 140 bpm as abnormal is quite stringent. Would you explain your reasoning for these criteria?

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DR. JOHN E. PATRICK, London, Ontario, Canada. I guess I probably reflect the feelings of most of us who have to go home and deliver high-risk pregnancies.

After hearing this presentation, I find myself asking, what should we as clinicians do? How do we use vital testing in our management of the high-risk pregnancy given your results?

DR. KEN NOLLER, Rochester, Minnesota. How did you select the women for inclusion in your study? Was this a group of normal women who were picked at random? Did they agree to the study?

I particularly wonder because since they had all been scheduled for induction, they could be women who were mostly at advanced gestational age, for example 42 or 43 weeks. The results then might not be predictive of women who were normal.

DR. HAROLD SCHULMAN, Mineola, New York. I wonder if this was a little bit of a statistical aberration. For example, if you had a larger series and a larger number of smaller for gestational age fetuses or those who had difficulty during labor and then selected that group and

saw what the predictability was, would we be seeing different results?

Dr. Low (Closing). The study population included patients admitted for induction of labor irrespective of the indication as long as they would consent to the protocol of the study. Two hundred twelve were classified as high risk and 78 as low risk based on selected antepartum risk criteria. Some of the low-risk patients then had intrapartum risk criteria.

This represents a population characteristic of many of the studies previously reported.

In respect to Dr. Schulman's question, we have looked at these associations in patients with long and short labors, in patients with and without antepartum risk factors, and in patients with and without intrapartum risk factors with the same results.

I agree with Dr. Resnik's comments. The need for a longer period of observation to use reactivity based on accelerations has been well demonstrated. The difficulty is the problem identified by Dr. Paul. There is a time implication if this test is to be used as a screening tool. As previous investigators, we selected 40 minutes as a compromise.

I would like to speak to Dr. Patrick's question. This is what really led us to this study. Any test that focuses our attention on the pregnancy at risk is positive. Most prospective studies of nonstress tests have low mortality rates. One of the variables is the degree of attention these patients receive.

Antepartum fetal heart rate is a noninvasive measure of the fetal state that will be useful in time. However, the nonstress test as it is presently interpreted is in my judgment, and I am sure some will not agree, a disservice to current clinical practice.

I believe that the statement "a reactive nonstress test provides reassurance about fetal well-being" should be discarded. All prospective studies of nonstress tests include unexpected fetal deaths, occurring within 24 hours of the nonstress test. Any busy obstetrician can provide anecdotal experiences of a fetus that has died within 24 hours of what they have observed to be a reactive nonstress test.

I agree with Dr. Resnik that it is time that we use tests for specific diagnoses. It is too much to ask of a single test that it identify all the fetal problems that may lead to morbidity or death. Where we have had a specific test for a specific diagnosis (Rh isoimmunization is one of the best examples), the results have been excellent.

Antepartum fetal heart rate should be interpreted in a way that will give it greater credibility, in spite of the fact that we lose sensitivity by so doing. There are several options in regard to the diagnosis of fetal hypoxia in the mature fetus.

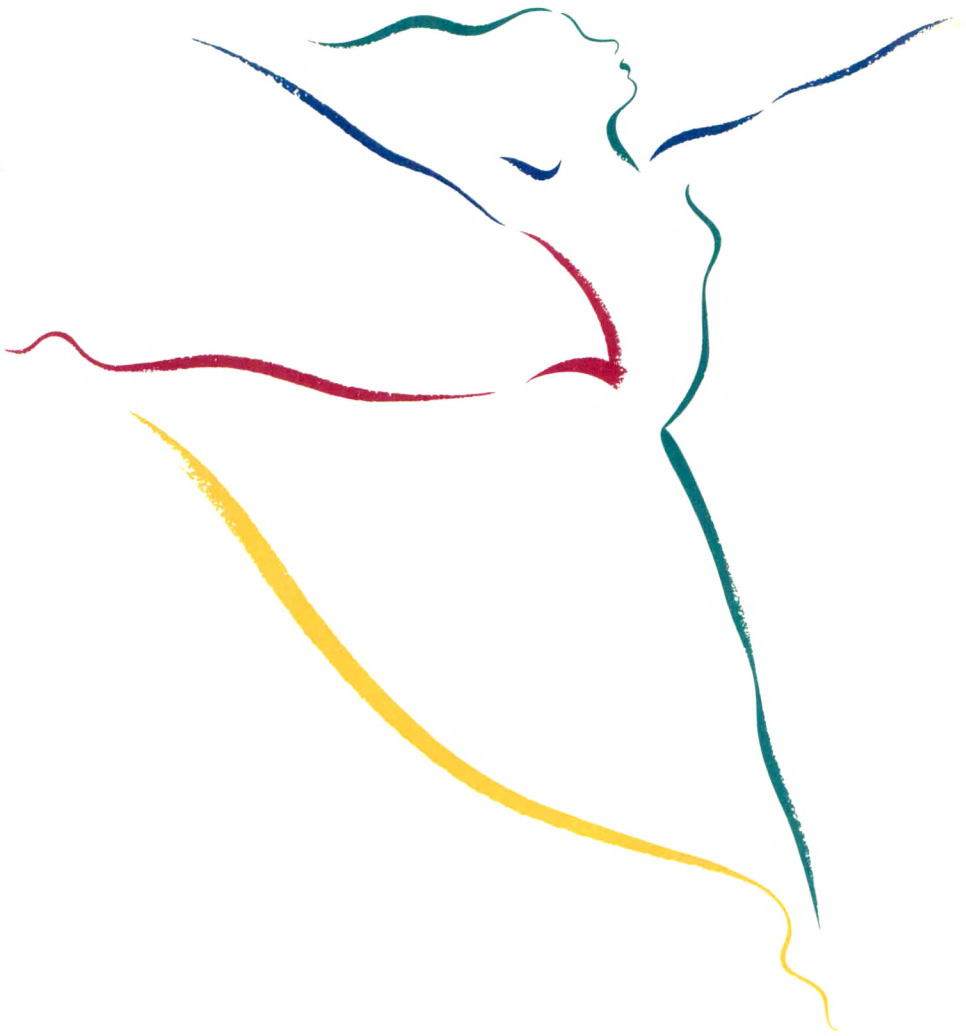
The first is to extend the period of observation from 80 to 120 minutes. The nonreactive test at this point will be associated with a low false positive rate.

The second is to use the composite score within a 20- or 40-minute period. There are several points that support this approach. One of the problems is the interobserver variability in regard to the observations used in the nonstress test.

Our interobserver reliability ranged from 0.5 to 0.9, being low for baseline variability and decelerations. If we use a test that requires more stringent interpretation, we will get a better analysis of the record. Second, the animal studies emphasize that for the specific diagnosis of hypoxia, decelerations are probably more useful. Finally, Kierse and Trimbos (see reference 5) have demonstrated a low false positive rate with composite scores of 5 or less. If we wish to have a more creditable test, we need to use more stringent criteria and accept the decreased sensitivity of the test at the present time.

The final option, of course, is to pursue the investigation of the fetal state to find a measure that is more sensitive for this specific diagnosis.

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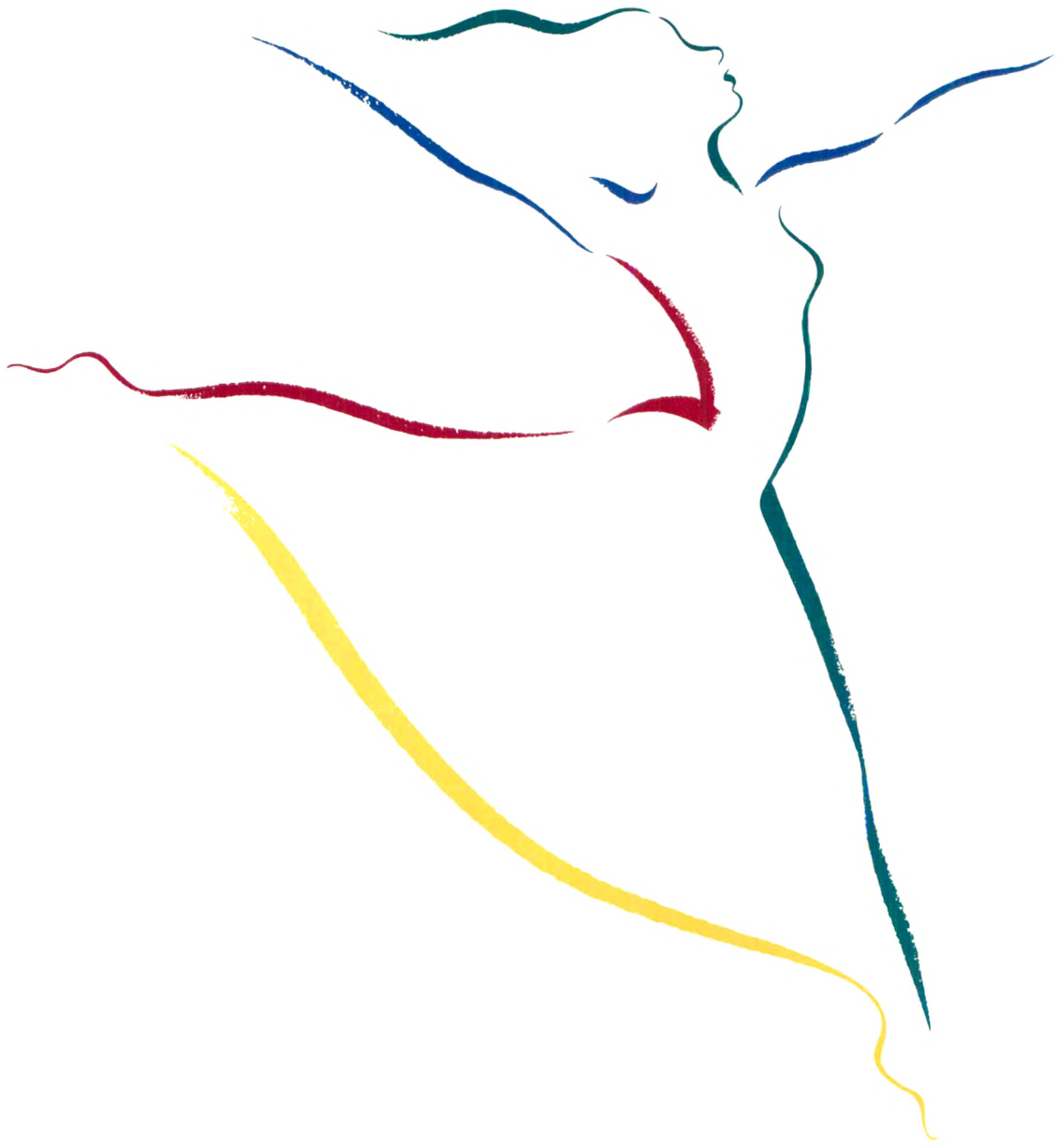
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1. According to a 12-month nationwide industry audit (ending October 1985), comprised of approximately 2,000 pharmacies. Vulvar burning occurred in 2.3% and itching in 0.9% of patients on Femstat.
2. Bradbeer CS, Mayhew SR, Barlow D: Butoconazole and miconazole in treating vaginal candidiasis. *Genitourinary Med* 61:270-272, 1985. (Study conducted in non-pregnant patients.)

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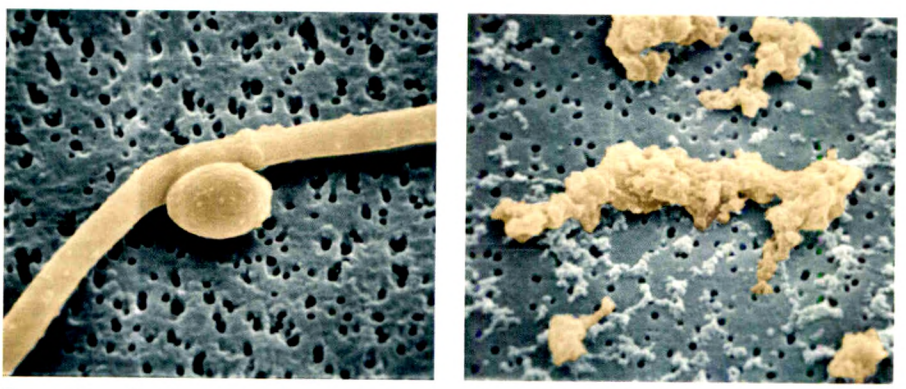
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Mutagenesis:
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Impairment of Fertility:
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Pregnancy Category C:
Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:
Use with caution.

Pediatric Use:
Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

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Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

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December 1985

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Symposium on the need for flexibility in academic obstetric-gynecologic residencies

Flexibility in obstetric-gynecologic residencies: Why is it necessary?

Introduction

J. Robert Willson, M.D.
Albuquerque, New Mexico

In his presidential address before this Society in 1983 Dr. Edgar L. Makowski identified several major problems that confront teachers, investigators, and clinicians in our discipline. These included resident education, the number and qualifications of clinical-scientists, the need to make the public more familiar with our mission and more involved in supporting it, and finally, the care of the elderly and the poor. He appointed a committee consisting of Drs. Saul B. Gusberg, James M. Ingram, Richard F. Mattingly, George W. Mitchell, Jr., and Henry A. Thiede, with Dr. J. Robert Willson as chairman, and charged them to ". . . seriously examine and deliberate the issues and . . . to formally convey its recommendations to Council . . . for subsequent action."

Several of the issues are of particular concern to the American Gynecological and Obstetrical Society because it occupies a unique position among the societies in our discipline. It is the only one in which the basic responsibilities of the majority of Fellows are the teaching of medical students, the preparation of residents for practice, the development of academicians, and the administration and operation of personal and departmental research programs. Other of the problems mentioned by Dr. Makowski, for example, the care of the elderly and the poor, although important, require more resources for their study and solution than can be made available through the American Gynecological and Obstetrical Society. We chose therefore to concentrate on issues that were particularly pertinent to our endeavors.

The committee members recommended that the most suitable projects for the American Gynecological and Obstetrical Society to consider were the development of objectives for resident education in university programs and of methods for monitoring the progress of house officers toward meeting the objectives; the development of standards for preparation of residents, Fellows, and faculty members for academic careers; and the identification and recruitment of lay persons who could interface with governmental bodies, agencies, foundations, and institutions in behalf of university departments. In addition we suggested that we offer to assist the Residency Review Committee and The American Board of Obstetrics and Gynecology in developing guidelines for approval of more than one kind of resident experience in selected university programs and that we offer to assist other organizations, such as the Council on Resident Education in Obstetrics and Gynecology, in developing criteria for the appointment of a program director. Finally, we recommended that the American Gynecological and Obstetrical Society develop a forum in which those involved with university programs could discuss the issues raised by Dr. Makowski and consider methods for solving them.

Dr. Joseph Seitchik, who followed Dr. Makowski as President, directed a second committee, made up of Drs. Jack N. Blechner, Philip DiSaia, William Droegemueller, and Charles B. Hammond, with Dr. J. Robert Willson as chairman, to review the original report and to recommend action that would be appropriate for the Society to initiate.

We concurred with the observation of the first committee that it is much more difficult now than it was in the past to develop and maintain the highest quality resident education programs and to recruit and train academic faculty. The principal problems are that there is no general agreement as to how training in a university department should differ from that in a community hospital, that there is no effective way to evaluate the progress of house officers as they advance from year to year, that it is difficult to vary the length or content of standard residency programs to accommodate special individual needs, that financing for programs designed to prepare residents and Fellows for academic careers is less readily available, and that it is difficult to support young faculty members as they establish their own research programs. It also was obvious to us that requirements for appointment as program director, even in university residencies, are much too permissive.

We recommended the following: (1) that the President appoint a Committee on Graduate Education to study and evaluate the reports of the two Committees

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and to recommend action steps that might be appropriate for the Society; (2) that a morning session during each annual meeting be devoted to discussion of a specific topic that concerns academic departments; (3) that the members of the Committee on Graduate Education and of the Program Committee cooperate in selecting the issue to be considered and in developing the program; and (4) that knowledgeable individuals, not necessarily Fellows of the American Gynecological and Obstetrical Society, be invited to prepare position papers to be presented and discussed during the session and that the papers and discussions be considered for publication with the scientific papers.

We also recommended that the first such session be developed for the 1985 meeting and that the subject be "Flexibility in obstetric-gynecologic residencies: why is it necessary?" Obviously, the Council approved the suggestion.

Finally, I should like to thank the members of both Committees who worked so diligently and effectively in gathering information on the issues and for their assistance in preparing the final reports. I also thank Drs. Jaffe and Hendricks for agreeing to prepare and present the papers today and to Drs. Merrill and Pitkin for their responses. They obviously share the concerns of the committee members.

The need for flexibility in preparing clinician-scientists for academic careers

Robert B. Jaffe, M.D.

San Francisco, California

Academic obstetrics and gynecology is not realizing its potential.

With reproductive sciences at the cutting edge of contemporary biology, and with increased numbers of outstanding, highly motivated and well-educated physicians choosing careers in obstetrics and gynecology, the discipline is not attracting a commensurate increase in the numbers of these highly qualified individuals into the academic research component of the specialty. The reasons for this are many and varied. They include: (1) lack of sufficient, appropriate role models during house officer training; (2) a negative perception of the competitive and political aspects of academic medicine; (3) the discrepancy in income between academic medicine and private practice; (4) inadequate exposure to, and hence inadequate appreciation of, the intellectual excitement of academic investigative pursuits; and (5) inflexibility in the training programs in obstetrics and gynecology in most academic centers. It is these latter two interrelated factors that will be the focus of much of this discussion. Although the majority of comments will be directed toward residency training, the discussion will be extended briefly to a consideration of the need for flexibility in fellowship training as well.

In many of the constituent disciplines of reproductive medicine and biology, reproductive science is, or should be, at the forefront. For example, in the field of endocrinology, over half of the articles in the two leading American endocrinologic journals deal with some facet of reproductive endocrinology. In the field of oncology, in which studies concerning normal and abnormal cell and tissue growth, oncogenes, and other aspects of cell biology have captured the imagination of fundamental biologists, gynecologic oncology has the opportunity to make meaningful strides. To a great degree, this potential has not as yet been realized.

There are no academic obstetrician-gynecologists currently in the National Academy of Sciences, only three in the Institute of Medicine, and just two in the Association of American Physicians, and tellingly, none are members of the American Society for Clinical Investigation, the "Young Turks," who are under 45.

In part because of (1) the excitement of contemporary reproductive biology, (2) a concern with important social issues, and (3) the attraction of being involved with both medical and surgical aspects of a discipline, as well as for a variety of other reasons, obstetrics and gynecology programs are attracting some of the brightest and best graduates of leading medical schools. Many of these individuals have had extensive research experience before beginning their house officer training. Yet relatively few pursue meaningful, academic investigative careers following their training. Of individuals who started Board-approved subspecialty fellowships that could have been completed before 1984, approximately 60% have full-time academic appointments. However, the number competing successfully for National Institutes of Health grants is very low compared to other academic disciplines. For example, in fiscal years 1983 and 1984, approximately 10% of National Institute of Child Health and Human Development extramural funding went to Departments of Obstetrics and Gynecology. Approximately half of the awardees held M.D. or M.D. and Ph.D. degrees. Thus about 5% of the awards of the National Institute of Child Health and Human Development and only 6% of its funds went to obstetrician-gynecologists. Of the total National

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Institutes of Health extramural budget, only 0.8% went to obstetrician-gynecologists.

Although various forms of scholarship are essential, it seems clear that there needs to be greater emphasis on productive research as an integral part of a greater number of academic departments of obstetrics and gynecology.

Several barriers exist for the pursuit of such research training. They include lack of significant exposure to contemporary research for the 4 years of residency training in many programs; a lack of first-hand experience in research by many of the leaders in obstetrics and gynecology; the presence of role models during residency training who spend most of their time in the clinical practice of obstetrics and gynecology; the additional time that would be required to satisfy subspecialty board requirements; and, for the individual with a strong research background, the 4-year period during which it is extremely difficult to keep current in and excited by contemporary investigative biology.

For these reasons we proposed several years ago an alternate residency training program for selected individuals who were contemplating academic careers and who had significant previous research experience. This program, in which at least two individuals would have to be contemporaneously enrolled for logistic purposes, would intertwine clinical training and research during a 6-year period. It would involve primarily clinical residency training during the first 2 to 3 years following medical school. Thereafter, a year would be spent in the laboratory and, in the case of reproductive endocrinology, in gaining expertise in the clinical aspects of endocrinology and infertility. After this the individual would complete his or her clinical and research training in a manner sufficient to qualify both for the general boards in obstetrics and gynecology and, for those areas in which subspecialty boards are offered, in those subspecialty areas as well. Although there is a limited number of centers in which such a program could be established now, this number can and should be expanded if the model is successful.

In my view the advantages of such a program are several: (1) the individual could stay in closer contact with the scientific aspects of the discipline without having to wait 4 years to renew that contact; (2) he or she would be guaranteed funding in advance for the fellowship experience as well as the residency; (3) research projects could be begun which could be continued during the remainder of the individual's training period; (4), and perhaps most important, the individual would have continued stimulus to pursue an investigative academic career.

The disadvantages to such a program include the following: (1) difficulties in scheduling rotations that would integrate these individuals with residents pursuing traditional 4-year training programs; (2) loss of some continuity in clinical training and experience; (3) decrease in the development of the camaraderie that occurs as an individual goes through training with the same group of individuals; and (4), unless these are additional positions rather than just substitute positions, antagonism and jealousies by other house officers. Although these disadvantages are real, in my view the advantages markedly outweigh the disadvantages.

However, the parent American Board of Obstetrics and Gynecology has not seen fit to give approval to such a program other than on a case-by-case basis. This is not feasible, since an applicant needs to know in advance, before making a decision about the location of his or her training program, that such an option would be available. In addition, program directors have to make decisions in advance so that they can plan appropriately for their training programs. It would seem that just as programs are approved for specialty and subspecialty training, selected programs also could be approved for this alternative form of training.

Two examples might be illustrative. Our house officer training program has had two individuals in it, both of whom had similar backgrounds and both of whom showed excellent promise for academic careers. Both had pursued M.D.-Ph.D. programs in medical school and had obtained their Ph.D. degrees in one of the foremost molecular endocrinology laboratories in the country. The first of these individuals, who completed his training 3 years ago, went through the conventional residency training program. He performed extremely well and, for several reasons, entered private practice at the conclusion of his training. His mentor in the laboratory in which he obtained his doctorate said that he was one of the most promising graduate students he had ever trained. While he is performing in an excellent fashion in clinical practice, there is a high likelihood that he also would have had a brilliant career in academic obstetrics and gynecology. The second individual, with a similar background, is the first to be pursuing the modified program which I described previously. On completion of his fellowship training, he will enter a faculty position in which, I predict, he will have a very productive career. Although there is no assurance that the first individual would have elected an academic career were he to have enrolled in the alternative pathway, it is my view that this would have enhanced the chances of his doing so.

Since there is a great need for exciting, well-trained clinician-investigators in academic departments of obstetrics and gynecology throughout the country, and since traditional training programs do not appear to be meeting this need, this alternative track seems worthy of trial in selected institutions.

With regard to fellowship training, it appears that

greater flexibility also might be beneficial if individuals are to be prepared for productive, academic research careers. Many of the current obstetric-gynecologic leaders in reproductive research pursued training programs that would be proscribed by current guidelines of the subspecialty boards. Thus, while many spent 1 or 2 years in a laboratory either abroad or in a basic science department in the United States, these are not components of "approved" programs of the current subspecialty disciplines. Furthermore, extensive clinical requirements, for example, the need for reproductive endocrinologists to train in tubal microsurgery and in vitro fertilization, and the large amounts of time in clinical pursuits mandated in fetal and maternal medicine and oncology mitigate against obtaining the requisite training to pursue an independent, productive, and competitive research-oriented career—and the discipline sorely needs this facet of the specialty better represented.

Although I was not personally involved, the activity of this Society of which I have been most supportive and proud has been the creation of the Kennedy-Dannreuther fellowships. They furnish tangible evidence of the Society's commitment to training investigators in the reproductive sciences. To maximize these opportunities, the greatest possible program flexibility needs to be encouraged and facilitated.

Experience with flexibility in an academic residency program

Charles H. Hendricks, M.D.
Chapel Hill, North Carolina

During my tenure as Director of the Residency Program in Obstetrics and Gynecology at the University of North Carolina at Chapel Hill, from 1968 through 1980, seven principles served as guidelines. All of these principles are important, but the first two were absolutely critical in our residency management during those years:

1. The residency exists primarily to serve the developmental needs of the resident and not the service needs of the hospital where he is being trained.

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2. Education is not neatly confined within the white picket fence of any institution nor tidily contained within a classical school year. The University should be thought of as a universal idea rather than as an institution. The strategy of utilizing the University should allow for rearranging the boundaries of time and geography where such adaptations can aid in the development of a single individual's career.

3. A residency should be considered to be a vital, demanding, prolonged postgraduate academic experience. Treating a residency training program as a trade school program demeans both the resident and the residency.

4. Ideally, residency experiences should be designed to help the person reach toward his highest aspirations for his own development. It is unrealistic to design a residency program to produce only academicians or only practitioners. Having high aspirations for a full-time academic career is no more worthy than having high aspirations for a successful career in private practice.

5. The individualization of resident care is just as important as the individualization of patient care.

6. Both the resident and the residency benefit by having some input by the resident, in the design of his own learning program.

7. It is manifestly impossible for the resident to "learn" obstetrics and gynecology during a 4-year period. Rather than "learning" the field, the resident should be learning intellectual inquiry and disciplined study methods that will qualify him as a life-long student in his chosen field. Only in this way can he hope to avoid boredom, obsolescence, and professional decay.

Strategies

The residents were selected through the usual selection mechanisms except for those who joined the program at an irregular time. We made provision for accepting as early starters, and therefore early finishers, those individuals who had completed their medical school requirements for the M.D. degree sooner than their fellows had done. During 12 of the years under study here, the resident applicants were encouraged to have some input into their own curriculum, designed to advance their personal goals. Their elective programs were determined partly by their requests and partly by suggestions offered by the faculty.

There was no requirement that any resident would have to perform any research. We did take care, however, to provide opportunities for research, which we enthusiastically aided and abetted.

If one applies seriously and logically all of the precepts listed above, it is perfectly apparent that there

Table I. Graduates of the University of North Carolina residency program by years, 1970-1982*

Year	*No. of graduates*
1970	4
1971	3
1972	2
1973	2
1974	4
1975	5
1976	5
1977	5
1978	3
1979	6
1980	4
1981	5
1982	5

*Mean number of graduates per year, 1970-1974: 3.0. Mean number of graduates per year, 1975-1982: 4.8.

will not necessarily be the same number of residents within the home hospital training department at any one time. There will be a great deal of coming and going of residents as they go from one assignment or elective to another in pursuit of the individual 4-year plan. Our training program needed to make allowances for the fact that the training was not even necessarily continuous. For example, when two of our residents dropped out after 2 years to work at the Centers for Disease Control, they returned to finish their final 2 years at the University of North Carolina. Three others joined the program at the second-year level.

The total number of graduates did not vary widely from year to year, since some residents transferred quite early to other activities. Altogether, 65 physicians entered the training program. Of these, 11 (17%) departed for other activities; only one of these was with our program more than a single year. Two entered required military service; after completing the military requirement, one of these went into family medicine, while the other completed the obstetrics and gynecology residency in another program. After no more than 1 year in our program, one resident left for further training in each of the following specialties: pathology, psychiatry, radiology, radiation therapy, ophthalmology, anesthesiology, pediatrics, and family practice. One resident departed after 2 years to enter the Public Health School.

The planned number of residents during most of the years was five per year (Table I). Actually, with one exception, in only one year was that number exceeded: in that year, there were six graduates. It should be noted, however, that in the year just preceding and the year just following that year, the actual number of graduates were only three and four, respectively.

Table II. Types of electives chosen by graduates of residency program

	On campus	*Off campus*	*Total*
Research	8	2	10
Oncology	4	6	10
Extra clinical	1	8	9
Surgical specialties	3	6	9
Maternal/fetal medicine, including genetics and ultrasound	5	3	8
Endocrinology	1	5	6
Pathology	5	1	6
Anesthesiology	1	4	5
Other	6	6	12
Total	34	41	75
Percent	45.3%	54.7%	

In general, we abided by the guideline that our residents would have 3 years of categorical rotations but not necessarily serve those rotations in any required identical order. This left 12 months for elective experiences. The elective experiences were expected to be worthy of a graduate study level, to be challenging, and also to be building blocks for the long-range growth of the individual.

In the preparation of our report on the residents' experiences, the resident graduates were surveyed. During the inclusive years 1970 to 1982, 53 residents completed our program. An additional one who finished a year later is included because he had been away for 2 years at the Centers for Disease Control and thus was a late finisher. The graduates were asked to react to their residency experiences in the light of what they have learned since they have left the residency. They were also asked to describe their current activities, their curricula vitae, and their future plans. Of the 54, 45 (83.3%) responded. The following report draws heavily on their replies and perceptions.

Stresses in a flexible system

Of the 54 graduates the great majority indicated their approval of the flexibility features.

However, a minority of graduates were critical of the elective system for various reasons. One said he had regrets at having failed to sign up for any electives. One expressed criticism of the program for having failed to apply pressure on him to seek elective experiences.* One superb and stalwart graduate said that he had no opportunity to sign up for electives because he was too busy carrying major patient care clinical loads, which

*My own recollection is that a large proportion of the residents were reluctant to sign up for any elective that they feared would dilute their categorical-type training experience!

Table III. Off-campus training sites for electives within the United States

Training site	*Area of study*	*No. of residents*
Atlanta—Cullen Richardson	Gynecologic surgery	2
Bowman Gray	Anesthesia	2
Case Western Reserve	Anesthesia	2
Columbia	Pathology	1
Duke	Endocrinology	2
George Washington	Surgery	1
Jefferson	Endocrinology	1
Mayo	Gynecologic surgery	2
Texas/Southwestern	Maternal/fetal medicine	1
University of California (Los Angeles)	Urology	1
Sloan-Kettering	Oncology	3
Centers for Disease Control		2
Total		20

indeed he was. Two believed the electives were not evenly distributed. Several found the frequent coming and going of residents and the variability of the size of the resident staff on duty at any one time to be unsettling.

One graduate provided the following insightful commentary on what a flexible program meant to him. "I believe UNC's graduates are able to consider alternatives to diagnosis and treatment because we had time and were encouraged to discuss problems rather than follow a 'cookbook'. . . . It remains imperative that UNC continue to choose residents that can handle this pressure of being more independent than residents of other programs . . . . The flexibility of our program is an excellent concept as long as residents or at least the majority of them are able to handle the stress and anxiety of accepting the responsibility that comes with flexibility."

Some positive feelings

Here are excerpts from other graduates' reports:

"The flexibility of the program at UNC allowed and encouraged me to function as an adult. My assessment is that many residency programs currently treat residents as children, rather than 26+-year-old individuals who have a good idea about what they need and want in order to grow personally and professionally. I would suspect that you will receive an argument against a flexible residency program—the statement that this sort of program would only work for a certain small segment and subset of the total group of GYN residents. While this may to some extent be true, I think the encouragement of self-motivation, assessment of character needs, and goals coupled with the guidance when needed from attendings within the basic structure of residency is the optimal situation."

". . . the program at UNC provided me with invaluable academic contacts and started academic habits . . ."

"The ease with which alternative educational activities blended with the program, the flexibility both with regard to internal and extramural scheduling, and the sincere (not just lip service) support and encouragement to broaden educational experience were unmatched by any program I interviewed at or have seen since. It still remains an ideal goal for programs I am involved with now."

"While I believe that the flexibility which you built into the program was exceedingly important, at least equally important in my mind was your strong support of the residents as they sought to take advantage of the opportunities you helped to create."

". . . the residency experience was more weighted to postgraduate education and less to technical training than other programs with which I am familiar. Nonetheless, I gained a good basic experience in clinical obstetrics and gynecology, such as surgical techniques and endocrinology, which has enabled me to continue my evolution as a specialist in infertility treatment, and which allows me to apply the skills in preventive medicine and epidemiology which I have also continued to pursue since the residency years."

"I am aware and will always be grateful that you squeezed me in after your five first-year slots were filled (to my chagrin!) . . .. The experience was freewheeling and intellectually exciting. You assembled a critical mass of superb people!"

Elective activities

Overall, the residents described 75 elective experiences that were meaningful to them. Thirty-four of these (45%) were electives undertaken on the campus of the University of North Carolina, and 41 (55%) were taken off campus (Table II).

A wide latitude was provided. Most commonly, the electives chosen tended to be grouped within a relatively few areas. Over half of the experiences described focused on five areas. Ten residents had one or more oncology electives. Ten had research electives, nine had surgical specialty electives, and nine had extra hands-on clinical electives. Eight chose electives relative to maternal and fetal medicine, including ultrasound, genetics, and neonatology. The remainder of the 75 electives varied widely in content and objective.

I will cite five unique electives: (1) A resident joined the Division of Medical Education for 3 months, as preparation for a career in academic medicine. (2) A resident established a university sex education course, which attracted wide interest and imitation. (3) Another resident established our departmental colposcopy clinic. (4) The same resident computerized our oncology records, a most demanding task but most produc-

Table IV. Off-campus foreign elective sites

Foreign elective site	*No. of residents*
Jamaica—Project Hope	4
Stockholm—Karolinska	3
Oslo—Norwegian Radium Hospital	1
London—University College Hospital	1
London—King's College Hospital	2
Vienna	1
Israel	1
Total	13

Table V. Memberships and certification of graduates of residency program

	n	%
Listed in Directory, American College of Obstetricians and Gynecologists	51/54	94.4
Diplomates of American Board of Obstetrics and Gynecology*	51/54	94.4

*Two more ready to take Board examinations for the first time after required waiting period.

tive in his future career. (5) Another resident undertook a heavy reading and study course during her 10-week maternity leave. This resulted in an article describing her own physical and emotional response to the pregnancy and delivery process. Publication of that article in turn led to multiple invitations for this graduate to participate extensively with women's education.

There were 20 elective experiences conducted at 12 off-campus sites in the United States (Table III). These included service at nine medical schools. Also included were three Galloway Oncology Fellowships at Sloan-Kettering, two 2-year stints away from the residency at the Centers for Disease Control, and two gynecologic surgical rotations in Atlanta.

Thirteen of our residents elected to do clerkships abroad (Table IV). Four of these were served at the University of West Indies in Kingston, Jamaica, as part of our Departmental participation in Project Hope. Three electives were served in London (one at University College Hospital and two at King's College Hospital), three at the Karolinska Institute in Stockholm, and one each in Vienna and Israel.

It appeared that prolonged furloughs or other absences from our campus did not deter the development of careers—in fact, it was quite the contrary with the Centers for Disease Control. Two of our residents had 2-year furloughs to work there, and a third joined our program at the completion of his 2-year appointment there. In each case the Centers for Disease Control proved to be an exceptionally stimulating and productive environmental influence. The resident who spent a year in Sweden learned research techniques that have helped his academic career.

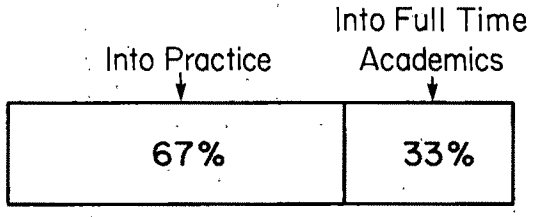


Fig. 1. Percentage of the graduates who went directly into private practice and those who entered full-time academic departments.

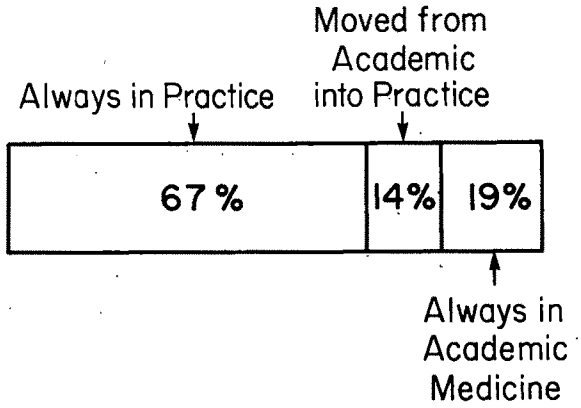


Fig. 2. Percentage of those graduates who went directly into practice, those who left academic departments to enter private practice, and those who have remained in full-time academic departments.

Outcomes of a flexible program

It is difficult to quantitate precisely the effect that flexibility and versatility in educational endeavor have on the final product, the graduate of such a residency program. As a general indication of overall results, I have elected to report on the following: membership and certification, a description of activities since leaving the residency and their relationships to medical school teaching programs, and evidence of scholarly activities as indicated by publications listed on curricula vitae.

Membership and certification. Of our 54 graduates, 51 are listed in the most recent directory of the American College of Obstetricians and Gynecologists. Fifty-one are Diplomates of The American Board of Obstetrics and Gynecology, with two more waiting to take Board examinations for the first time as soon as they are permitted to do so (Table V).

Four of our residents used elective opportunities in partial fulfillment of the Board requirements for specialties other than obstetrics and gynecology (Table VI). One resident had a 5-month elective in obstetric anesthesiology. At the completion of his formal residency, he achieved additional experience and ultimately became a Diplomate of The American Board of Anesthesiology. A second resident, having already served an internship in internal medicine, chose to use his 12

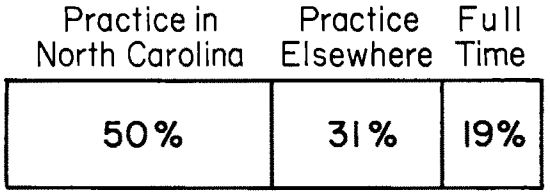


Fig. 3. Location of graduates in private practice or in full-time academic medicine. One-half of the graduates are now in practice in North Carolina.

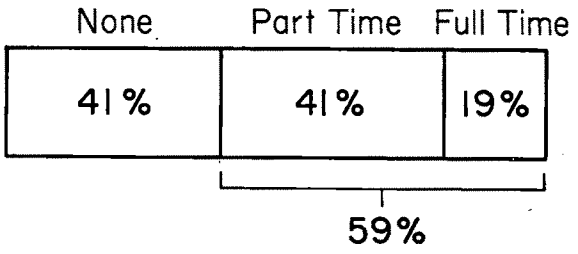


Fig. 4. Nearly three-fifths of the graduates now hold full-time or clinical teaching appointments.

months of electives entirely for internal medicine rotations, thus completing the formal training requirements that ultimately allowed him to become a Diplomate of The American Board of Internal Medicine. A third resident, having interned and then served a year of residency elsewhere that was followed by 2 years at the Centers for Disease Control, joined our program as a second-year resident for the final 3 years of his training experience. For his elective work he chose to obtain a Master of Public Health degree in our Public Health School, which ultimately helped him become a Diplomate of The American Board of Public Health and Preventive Medicine. A fourth resident also achieved a M.P.H. degree during his residency and was also able to become a Diplomate of the American Board of Public Health and Preventive Medicine.

Activities after leaving the residency. Of the 54 graduates, 36 (67%) went directly into private practice, while 18 (33%) went into full-time academic careers (Fig. 1). Of these, 16 were in medical school academic programs and two became permanent officers at the Centers for Disease Control, a career which, in my opinion, represents the equivalent of full-time academic activity.

With the passage of time, eight of those originally in full-time academic medicine moved from academic careers to full-time practice, leaving only 10 now in full-time academic careers (Fig. 2).

Where are they now? At present, 27 (50%) of the graduates of the program are in full-time private practice in North Carolina (Fig. 3). The primary mandate and mission of our training program was to produce well-trained specialists to serve the State, and it appears

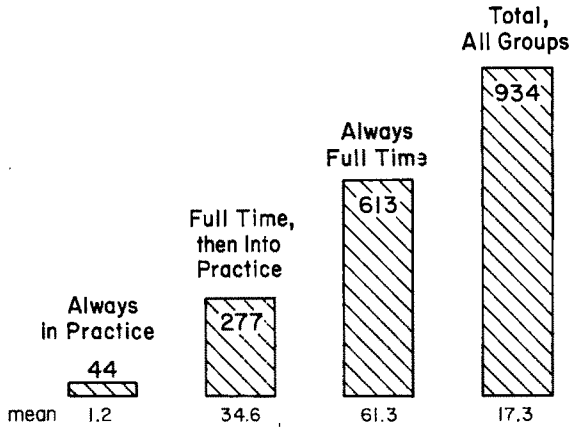


Fig. 5. Publications listed by graduates according to current professional activity. Of the 934 total listed publications, 613 appeared on the curricula vitae of the 10 full-time academicians.

Table VI. Certification by a Board in addition to certification by the American Board of Obstetrics and Gynecology

Additional Board certification	*No. of residents*
Public health and preventive medicine	2
Anesthesiology	1
Internal medicine	1
Total	4
Percent	7.4%

that this mandate has been well fulfilled. Seventeen (31%) are in private practice elsewhere, while 10 (19%) are still in full-time academic medicine

It is a matter of interest to note the current relationship between our 54 graduates and their teaching activities at various medical schools (Fig. 4). As mentioned, 10 are full-time staff members, but in addition to this, 22 (41%) hold part-time appointments for medical student and/or resident training in association with various medical schools across the country.* Thus 59% are currently performing teaching obligations in one of 12 medical schools. Four schools are in North Carolina: Bowman-Gray School of Medicine, Duke University, East Carolina University, and the University of North Carolina. The medical schools outside North Carolina are University of Arizona, Emory University, Hershey Medical Center, University of Mississippi, University of Nevada, Ohio State University, University of Oklahoma, and University of Oregon.

Publications. The publications to date as listed on

*The two Centers for Disease Control officers also have clinical appointments but are listed with the academic full-time quota and are not counted in the part-time teaching group.

Table VII. Publications listed on curricula vitae of the 54 graduates

No. of publications	*Always in private practice*	*Full-time academic, then private practice*	*Always full-time academic*	*Total*
Over 100		1	1	2
51-100		1	6	7
26-50	1	1	1	3
6-25	1	4	1	6
1-5	8	1	1	10
None	26			
Total	26	8	10	54

the curriculum vitaes submitted by the various graduates are shown in Table VII. With very few exceptions, all items submitted are listed, including abstracts, papers in journals, chapters, books, and a scattering of articles appearing in non–peer reviewed publications. The 36 residents who have always been in private practice list a total of 44 publications or a mean of 1.2 publications per graduate. The individuals who started out in full-time academic careers but then went into full-time practice have published a total of 277 articles, a mean of 34.6 per graduate. The 10 individuals always in full-time academic medicine list a total of 613 publications, a mean of 61 per full-time graduate (Fig. 5).

In conclusion, it should be stated that a program as flexible as ours was during the decade of the 1970s would not be applicable to most other American training programs. There may be some features of our approach, however, that might find limited usefulness in the 1980s.

Response on the need for flexibility in academic obstetric-gynecologic residencies

James A. Merrill, M.D.
Seattle, Washington

It is appropriate that the American Gynecological and Obstetrical Society be concerned with resident education in our discipline and that emphasis be on the production of individuals who will further education and research. This is not the first time such an issue has been addressed here. In 1953 the American Gynecological Society appointed a committee to study the problem of recruitment of academic personnel for the specialty of obstetrics and gynecology. The work of that committee lead to the publication in 1961 of a report entitled "The Recruitment of Talent for a Medical Specialty." Many of the observations and recommendations in that report remain valid in 1985, 25 years later.

Although the recent American Gynecological and Obstetrical Society committees have addressed many of the same issues included in the earlier report, they have concentrated their interests on graduate medical education and preparation for academic careers. The American Gynecological and Obstetrical Society committee has stated ". . . If we are to develop different tracks for those preparing themselves for academic careers, the policies of The American Board of Obstetrics and Gynecology and the Residency Review Committee must be adjusted to accommodate them." This morning's symposium is a discussion of that opinion.

Many years ago the program director of a prestigious residency program proposed that graduates of his program should be certified by the Board without the necessity of examination. He reasoned that his program was at least 2 years longer than standard residency programs and that residents were selected because of their talent and interest in academic medicine. Unfortunately, that same year, graduates from that program failed the oral Board examination. Whatever its other policies, the Board has always maintained that individuals in academic positions should be at least as competent and preferably more competent than others and that their education should be at least as comprehensive and preferably more comprehensive than others. Certainly, there can be no quarrel with providing outstanding house officers with the opportunity to develop skills *beyond* those needed in practice, but there may be argument about developing other skills *instead* of those needed in practice.

Let us look at Board policy and see where it needs to be adjusted to accommodate academic programs. The second American Gynecological and Obstetrical Society committee has arbitrarily defined an academic program as one with the capability of preparing house officers to practice as specialist/consultant obstetrician/gynecologists. This essentially is what the Board seeks to evaluate in *every* candidate for certification. The

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Board's definition of an obstetrician/gynecologist is "... individuals prepared to provide medical and surgical care for disorders that effect the reproductive system, the fetus or the newborn. These physicians have particular knowledge and skills which enable them to serve as consultants to physicians who practice in other areas of medicine." There may be a discrepancy between the ideal and the actual practice of obstetrics and gynecology. We must recognize the recurrent unanswered question of whether an obstetrician/gynecologist should be a specialist/consultant or a generalist with a "strong suit." The nature of the obstetrician/gynecologist has never been defined to everyone's satisfaction. For this reason, our approach to residency education is often schizophrenic—providing education neither for primary care providers nor for true consultants. However, the Board's definition seems to speak clearly to the question. To suggest different curricula or tracks for the basic preparation of academicians invites the development of different levels of certification.

The second American Gynecological and Obstetrical Society committee has identified five important factors that they believe prevent the development of high-quality residency programs and the recruiting and training of academic faculty. Of these, possibly three relate to the Board.

"1 . . . Academic programs have little opportunity to vary either the content or length of residencies to meet special or individual needs." Board policy prohibits neither. It does seem wise that modifications should be directed to expanding education, not contracting it, and that this should not be accomplished at the expense of uninvolved residents. Thus it may not be wise to mix curricula in the same program. The Board does have a policy regarding minimum duration of education but not maximum duration. Candidates must complete no less than 4 years, which must include at least 36 months' training in obstetrics and gynecology including a final year as Senior or Chief Resident. There is no prohibition against longer programs. Recently the Board has discussed lengthening the educational requirements. It has also discussed different levels of training and certification. There has not been great enthusiasm for either proposal but discussion continues.

"3 . . . There is no general agreement concerning the content of a program for developing specialist/consultant obstetrician/gynecologist and no specific requirements for graduation . . . " The Board is not directly involved in the content of residency programs, although it is indirectly involved in terms of the content of its examinations and the affidavit by which program directors attest to acquisition of certain knowledge and skills. The Board does agree that there is need to establish a defined base of knowledge and technical skills and is starting to address the issue of content. It is interested in finding out what currently exists in residency programs in terms of faculty, education, and training facilities. This information is not otherwise readily available. The Board is directly involved in both length and content of subspecialty fellowship programs which, of course, have as an important objective the preparation of academic faculty. The fact that the Board reviews and approves fellowships permits individualization and innovation more easily than can be accomplished in residency programs.

"4 . . . There is no effective method of evaluating the progress of house officers through such programs or of assuring the director that each has acquired the knowledge and developed the skills necessary to practice as a specialist." The Board recognizes this also as a problem for many programs. It is now in the process of developing a "pre-test" that will test for the basic information on which advanced knowledge and skills may be developed. This test may serve the purpose of providing program directors with helpful information necessary for the counseling, promotion, and graduation of residents.

Dr. Hendricks and Dr. Jaffe each have described an innovative approach to graduate medical education. Dr. Hendricks has described an individualized educational experience for residents, with an objective of serving as the basis for life-long growth of the individual, whatever his/her career. I do not see how this has been hampered by Board policy. His program has provided 12 months for electives. Within a 4-year program, as much as 1 year can consist of any sort of accredited education. He has used this opportunity to a maximum degree. His graduates have been eligible for Board examinations. Although there has been great "coming and going" of residents, the faculty at the University of North Carolina have managed to maintain an appropriate educational experience. The program has been successful, as have more rigid programs. This success is apparent, since essentially all graduates are Board certified, over half practice in North Carolina, a significant number hold full-time faculty positions, and the graduates have shown scholarly activity as measured by publications and medical school teaching assignments. Indeed, success may be greater than what Dr. Hendricks has reported, since it is difficult to measure all aspects of self-achievement. Thus this flexible program appears not to have been prevented by Board policy.

Dr. Jaffe proposes a modification of graduate medical education to encourage and improve research in this discipline. He considers this necessary because the academic area of obstetrics and gynecology is not realizing its potential. Approximately 3 years ago Dr. Jaffe made this proposal to the Board. After repeated

discussion, the outlined education was approved for one or two candidates. To the best of my knowledge the Board has not been notified that anyone has followed through with the approved curriculum. Dr. Jaffe is correct that the Board approved a program for individual circumstances and not categorically for the institution. The Board wished to observe the result of this experiment before authorizing it as a standard procedure.

The modifications in this proposal are quite different from those at North Carolina. This curriculum proposes to integrate residency and fellowship training and to do so by introducing some aspects of the fellowship training before completion of residency. Thus this proposal has its real impact on the policy governing fellowship rather than the residency. When this involves laboratory research, there is not likely to be a significant problem. However, when it involves clinical portions of the fellowship, there is reason for controversy. There has been a basic philosophy that subspecialization builds *on* basic knowledge and skills acquired during residency and not *instead* of them. Nonetheless, the Board has expressed a willingness to experiment with such a curriculum, recognizing that the research requirements of the three subspecialties are not similar. Indeed, the Board, in separate action, has accepted the proposal that extensive prior research experience may substitute in individual cases for 1 year of the required fellowship in reproductive endocrinology.

However, this sort of interdigitation between fellowship and residency, as well as any marked variation in resident rotation, raises the question of what should constitute the final or chief year of a residency program. Until recently, opinion has been that residency education should culminate in a time of independent decision-making and patient management and should be the final step in progressive acquisition of skills and knowledge. There may be reasons for change. The old pyramid system was dropped. If we are prepared to recognize a variation in the product of graduate medical education and in practice, then it might be appropriate to reconsider this system for some.

Although the Board may not subscribe readily to the concept of both academic and nonacademic programs, it is interested in any innovation that will improve the quality of education for obstetrician/gynecologists. Concern has been expressed that research in obstetrics and gynecology may have been diminished by the substitution of clinically oriented subspecialty training programs for the laboratory training that otherwise might have been pursued by those entering academic careers. Therefore, three studies were conducted by the Board, addressing research in obstetrics and gynecology. One was a study of scholastic ability of residents, another was the analysis of a questionnaire sent to everyone who had been in a subspecialty fellowship, and the third was an analysis of a questionnaire sent to all chairmen of medical school departments of obstetrics and gynecology. The response to the questionnaires was 71% and 81%, respectively.

The scholastic ability of those who finished obstetrics and gynecology residencies in 1983 and 1984 was studied. To the extent that scholastic ability is measured by performance on National Board of Medical Examiners Part I and Part II examinations, residents in obstetrics and gynecology, on the average, had slightly less of that ability than national norm groups who took the same examinations, a finding remarkably similar to the American Gynecological Society study reported in 1961.

Between 1974 and 1984 the mean number of full-time faculty members in departments of obstetrics and gynecology doubled. Ninety-three percent of medical school chairmen report trained subspecialists in one or more subspecialties, and 60% of them report trained subspecialists in all three areas. However, approximately one fourth of the departments indicate no research funding from the federal government, and one fourth of the schools report no research funding from foundations. Over one half of the responding department chairmen do not plan to recruit a candidate with an M.D. degree to spend 50% of his time in laboratory research during the next 5 years.

Sixty percent of prior fellow respondents have full-time faculty appointments, and 16% are in private practice. Approximately 50% of their professional time is devoted to the subspecialty. The mode and median time devoted to research is 10%. Most research is clinical research, and 57% of respondents do no laboratory research. Two thirds of the subspecialist respondents have never filed research grants with the federal government. However, of those who have submitted grants, 64% of the grants were approved and 34% of the grants were funded. One fourth have published no papers since completing fellowships.

I agree with Dr. Jaffe that academic obstetrics and gynecology is not realizing its potential. There are many factors impacting on this, not the least of which are financial. Many of the problems identified by Howard Taylor's American Gynecological Society Committee, 25 years ago, are still with us. Although I consider the attitude and priorities of department leadership the most important, innovation and flexibility in graduate medical education certainly are important. However, I do not believe that either is prevented or inhibited by Board policy. The Board may be somewhat rigid but not ossified! Tracks for academicians can be worked out, but they must not hamper other residents. Both Dr. Jaffee and Dr. Hendricks identified this as a potential disadvantage. There can be more than one kind

of residency training program so long as each is a good one. Rather, I think the limitation to innovation and flexibility in graduate medical education is in the imagination of our leaders and in the lack of a clear definition of the specialty. Exciting and imaginative training programs are difficult to conceive and difficult to administer. In 1955 the American Gynecological Society Research Subcommittee stated ". . . The nature of this specialty makes it well nigh impossible for the practitioner to have much consecutive time for reflection, reading or investigation and it is the genuine impression of this committee that our field has not in the past 25 years attracted its share of the scholars. It is entirely possible that the research status of our residents simply reflects the research activity of their leaders." The success of the residency programs at Chapel Hill and San Francisco and of many other fine programs may reflect not so much the flexibility and individualization of the curriculum, as it does the imagination and enthusiasm of the faculty.

Response on the need for flexibility in academic obstetric-gynecologic residencies

Roy M. Pitkin, M.D.
Iowa City, Iowa

Contained in the invitation to participate, on behalf of the Residency Review Committee, in this program on flexibility in academic residencies was the suggestion that my discussion include the "reasons for the presumed rigidity of the two accrediting bodies in considering requests for deviations from the basic requirements." I am not certain precisely what this means, but I assume no one would seriously suggest that there ought to be two sets of standards, one for residencies designated by some means as "academic" and the other for the rest, for such would clearly be in conflict with all traditions of fairness as well as with the constitutional provisions for equal protection and due process.

If, as seems more likely, the type of flexibility desired is that described by Drs. Jaffe and Hendricks, I wonder if the problem may not be more imaginary than real. In comparing each of these programs, one operative for the period 1968 to 1980 at the University of North Carolina and the other proposed for the University of California (San Francisco), with the current Special Requirements, I can find little if any intrinsic conflict. Let me cite a few examples.

Perhaps the most specific (and therefore the least flexible) of the Special Requirements is that relating to length of graduate medical education in this discipline: "A resident must have a minimum of 4 years of approved clinically oriented graduate medical education, of which at least 3 years must be entirely in the specialty of obstetrics-gynecology." Dr. Hendricks indicates that the general guideline at the University of North Carolina was for 3 years of "categorical rotations," leaving a year for electives; parenthetically, one wonders how often residents spent as much as a year on electives. Dr. Jaffe's proposal is not quite as specific, but I do not believe it is inconsistent with this requirement.

The Special Requirements are also quite specific about the senior or chief residency year, reflecting the Residency Review Committee's belief that this period represents the most important segment of the residency. It must consist of 12 months of clinical experience, and electives or extramural rotations are not permitted without prior Residency Review Committee approval. Neither Dr. Jaffe nor Dr. Hendricks addressed the senior resident year specifically and I wonder how their proposal would square with the present stipulations.

If clinical training is interrupted to send residents off for research or other elective experience whence they later return to reenter the clinical cycle, the program might find itself at times over its approved complement. But this risk also may be more theoretical than real, for Dr. Hendricks has shown that in only 1 year of 13 did the University of North Carolina program exceed its approved number of graduates and that year was flanked on either side by under-quota years.

The Residency Review Committee has in my judgment acted quite flexibly with respect to requests for a temporary increase of one in total resident complement. Such requests are considered by the Secretary and Chairman and are approved expeditiously as long as the program is not on probation, the clinical volume adequate to accommodate another resident, and the basis of the request reasonable. With respect to the last, an effort to facilitate a resident's desire for prolonged research experience would surely be regarded as reasonable.

In Dr. Jaffe's proposal, some trainees may interrupt clinical training for prolonged periods (for example, a year or longer) and then reenter, raising the potential for conflict with the Special Requirement that "programs of different lengths or quality in the same institution will not be approved." However, any problem can be circumvented easily if the individual during this

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Table I. Residents completing training from 1970 to 1982

	University of North Carolina	*University of Iowa*
No. of residents	54	66
Attrition (%)	17	3
American Board of Obstetrics and Gynecology certification (n)	51	64
Other Board/graduate degree (n)	4	5

time is not designated as a resident. Indeed, this seems to have been the practice at the University of North Carolina, for Dr. Hendricks speaks of residents being "furloughed" to the Centers for Disease Control.

Terms like "flexibility" and "rigidity" are, like beauty, actually in the eye of the beholder. Whereas the Residency Review Committee may be viewed by many as inflexible, I am unable to find any intrinsic conflict between the Committee's published standards and the concepts of flexibility put forth in these essays. I would expect that all could find agreement with the peroration of the Special Requirements: "It is not essential or desirable that all training programs be identical in structure or function. It is, however, mandatory that each residency program provide quality education and experience for each of the residents completing the program."

This concludes my response on behalf of the Residency Review Committee. I would like, however, to continue the discussion as an individual program director. Dr. Hendricks' description of the program he developed at the University of North Carolina spurred my interest in compiling similar data from graduates of the University of Iowa program during the same time (1970 to 1982). The two institutions are similar in many ways (state university, full-time faculty, university hospital base, small town, etc.). Moreover, I believe the Iowa faculty, led for the first half of that period by Dr. William C. Keettel, would endorse wholeheartedly at least six of the seven principles Dr. Hendricks enunciated in his preamble. There might have been some difficulty embracing the second principle, which concerns rearranging time and geography to help a single resident's career, for the Iowa curriculum during the period in question was an utterly rigid, lock-step arrangement by which all residents followed precisely the same assignment schedule. Thus it was rigid where the University of North Carolina program was flexible, and I thought the comparison might prove interesting.

My data are based on the 66 graduates of the University of Iowa residency program during the years 1970 to 1982. The vast majority (78%) of those responding to the questionnaire indicated that the lack of flexibility in the Iowa residency was a good idea, and most of the rest (16%) thought it made no difference; only 6% saw it as any disadvantage. Several commented to this point and one, a successful departmental chairman, stated "In my experience, students and residents tend to choose pleasant, nonstressful electives. A good example is the waste of time of some of the overspecialized electives for senior medical students."

Table II. Current status of 1970 to 1982 graduates

	University of North Carolina	*University of Iowa*
Full-time academic career	19	21
Private practice in state	50	38
Private practice elsewhere	31	41

Table III. Publications by 1970 to 1982 graduates

No. of publications	*University of North Carolina*	*University of Iowa*
>100	2	2
51-100	7	2
26-50	3	2
6-25	6	16
1-5	10	22
0	26	14

Table I compares certain aspects of the two programs. The only very striking difference is in attrition. Dr. Hendricks has indicated that the 54 University of North Carolina graduates of that period reflected 83% of those who started training whereas only 3% of the number who began the Iowa program failed to finish. All but two of the Iowa graduates are certified by the American Board of Obstetrics and Gynecology, and those two are 1982 graduates who recently completed 3-year subspecialty fellowships. Four University of North Carolina residents used their elective time to fulfill, partially or completely, requirements for other board certification and/or graduate degrees. Without any such option during residency, five Iowa graduates registered similar accomplishments following residency (certification in pathology and anesthesiology, graduate degrees in microbiology, education, and preventive medicine).

Table II summarizes the current status of graduates of the two programs. The proportions in full-time academic medicine and private practice, both inside and outside the respective states, are similar. Of private practitioners, 39% of University of North Carolina graduates and 38% of Iowa alumni hold clinical teaching appointments in medical schools.

The number of publications by graduates is summarized in Table III. University of North Carolina

graduates have been somewhat more productive than have Iowa alumni, at least as reflected in the number of highly productive individuals. As a result, the mean number of publications per graduate was 17 for North Carolina and 12 for Iowa. On the other hand, more Iowa graduates have published at least one paper. At Iowa, as at the University of North Carolina during this period, there was no requirement regarding research or publication.

This comparison of two residency programs with somewhat different philosophies regarding flexibility/rigidity of curriculum indicates no substantial differences in the outcomes measured. Perhaps what is at issue here is flexibility of *thought* rather than of *curriculum*. I agree wholeheartedly with Dr. Hendricks' educational imperative that "the basis for patient management should start with an analytic approach rather than simply a dogmatic one."

We have all heard the old axiom about many ways of skinning a cat. If one starts with an intact cat and uses any of several appropriate means of cat-skinning, then one will likely end with a skinned cat.

Discussion

DR. ROBERT B. JAFFE, San Francisco, California. Drs. Merrill and Pitkin are extraordinarily articulate and persuasive. Let me just ask them a specific question. If either of the two individuals whose curricula vitae and bibliographies I showed to you were to apply to my program next year, may I commit to them in advance the type of flexible program integrating clinical training and research which I have suggested?

DR. JAMES A. MERRILL. Yes.

DR. ROY M. PITKIN, Iowa City, Iowa. The Residency Review Committee does not review and approve individuals. It accredits programs. If your program is accredited, you can make an agreement with an applicant that he complete that program.

When the program comes up for review, it will be evaluated as a program but not as it might affect individuals.

DR. MORTIMER G. ROSEN, New York, New York. I listened to this program with a great deal of interest and enjoyment, since one of the reasons I went into this specialty was not to be a researcher but to be an educator, and I have since changed and have even become a little more flexible.

I think there was an emphasis on the words "flexibility" and "rigidity," and I believe that is misplaced activity. As I heard the discussions, what I was impressed with was the originality of the program directors and the interest of the discussants, all of whom had been residency program directors with different plans producing different programs.

Then I thought that today most programs are just struggling to survive, and indeed, residency programs are under a tremendous amount of pressure today to deliver patient care, to produce educators, and to produce future scientists. Part of my comments will reflect this more broad charge, because I am going to muddy the waters of this discussion.

As I have just been at Columbia for 8 weeks, I will point out to you that in that system, the residency program is in the hands of a junior individual who just completed a training program in July. The only person who is as junior in our educational program is the Director of the Medical Students Educational Program. Both are well motivated, but quite junior. This may reflect the interest of our system in education. However, if we want educational and training programs to be a bit more superior, then we will have to recognize their leaders and their commitments—or indeed the program directors will not remain in that academic pathway very long.

Dr. Merrill made a comment that I think we should make note of. It would appear that the quality of performance of the individuals whom we have trained over the last 20-some years has really not changed. Although we would like to see the quality of the trainees improve, these numbers which Dr. Merrill has quoted really do not reflect a trend. The data lead me to the next statement. While this is an educational discussion, I would hope that you will permit me this statement made as an individual who has had continuing National Institutes of Health grant support for many years. In my travels to various hospitals in the United States I have often been asked to look at their problems in obstetrics. Inevitably when I look at the problems in obstetrics, I open the surgical book and also look at the problems in gynecology. I learned after a while that it was not surprising to find that clinical patient care "problems" were endemic within obstetrics, also were present in gynecology, but indeed, not as easily recognized as noting a cesarean birth rate.

Thus to muddy the waters here, what I would point out is that while there is a problem in improving the quality and the number of people going into the academic system and into the research pathway, which I am quite dedicated to improving and increasing, I would also point out to you that the clinical practice of obstetrics and gynecology is having just as much difficulty, and that problem is also important. Rather than to provoke individuals to respond in individual ways, do not focus alone on one part of our system, because very frankly I think we are just as much in need of improving clinical patient care as we are of attracting investigators into the research field. That too needs some rethinking.

DR. DENIS CAVANAGH, Tampa, Florida. I am sure, although we have some different interests, we all have a common goal, and the common goal is to improve the discipline of obstetrics and gynecology.

The first point is to have solid clinical residency programs. The American Board of Surgery has now decided that one has to have hysterectomies as part of the training before one can be certified in general surgery. This means that our residents are going to have to be

better in the operating room than they have been in the past. For this reason I agree with Dr. Merrill. We really have to have a solid core of residency training, on which to build the rest of our postgraduate education.

I think that the 3-year fellowships with a year in the laboratory is a very good plan, it being very important to orient the subspecialist as to the importance of scientific basis, whatever his/her career choice.

Third, I think that the Kennedy Foundation Fellowships really should be career awards. These career awards to truly distinguished and promising young people should provide sufficient financial inducement to keep them in academic medicine.

And fourth, we have to get busy and gain more influence nationally. The National Institute of Child Health and Human Development has just appointed a pediatrician again as the director, and we just heard that only 5% of the Institute's grants are going to obstetricians and gynecologists at this time. Is this going to get even worse?

The American Gynecological and Obstetrical Society and the American College of Obstetricians and Gynecologists (which has largely become just another union) really have to get into the act, and we have to get more influence at the National Institute of Child Health and Development and the other funding agencies in Washington, D.C. Without adequate funding for their research our young career scientists will become discouraged, and all our efforts to improve recruitment to academic obstetrics and gynecology will be doomed to failure.

The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome

John W. Goldkrand, M.D., and Armando M. Fuentes, M.D.
Albany, New York

Angiotensin-converting enzyme, the polypeptide that converts angiotensin I to angiotensin II, was measured in the serum of 114 pregnant women who had normal blood pressure, pregnancy-induced hypertension–preeclampsia, and chronic hypertension with or without pregnancy-induced hypertension. Angiotensin-converting enzyme levels were unrelated to weeks of gestation. The angiotensin-converting enzyme levels were similar in normotensive women (21.1 ± 6.9 units/ml), women with chronic hypertension without pregnancy-induced hypertension (23.1 ± 2.7 units/ml), and patients with pregnancy-induced hypertension where magnesium sulfate (22.6 ± 8.7 units/ml) had been administered prior to angiotensin-converting enzyme assay, but these values were significantly less than those in patients with pregnancy-induced hypertension with no magnesium sulfate (29.1 ± 6.5 units/ml) therapy and in women with chronic hypertension with superimposed pregnancy-induced hypertension (30.7 ± 4.4 units/ml) ($p < 0.005$). Maternal venous and umbilical venous and arterial angiotensin-converting enzyme levels were as follows: The maternal venous level was less than the cord venous level and greater than the cord arterial value. Neither neonatal size nor twin gestation influenced the angiotensin-converting enzyme levels. Patients with diabetes mellitus had variable angiotensin-converting enzyme values regardless of the status of the blood pressure. The physiologic theories of blood pressure control in pregnant women are discussed in relation to the renin-angiotensin, bradykinin, and prostaglandin systems. (Am J Obstet Gynecol 1986;154:792-800.)

Key words: Angiotensin-converting enzyme, pregnancy-induced hypertension–preeclampsia, chronic hypertension with or without superimposed pregnancy-induced hypertension, vascular responsiveness to pressor substances

". . . It is the increased responsiveness of the arterial system to pressor substances which probably causes the generalized vasoconstriction and hypertension of preeclampsia."[1]

Hypertensive disease of pregnancy complicates approximately 6% to 10% of all pregnancies and is responsible for increased maternal and perinatal mortality and morbidity. Investigations to define the pathophysiology of the pregnancy-induced hypertension–preeclampsia syndrome have as common factors the renin-angiotensin system and the vascular responsiveness to pressor substances. Angiotensin-converting enzyme is a polypeptide with a molecular weight of 140,000 daltons, which is secreted by the vascular endothelium and converts the decapeptide angiotension I to the octapeptide angiotensin II, one of the most potent vasoconstrictors.[2, 3] Abdul-Karim and Assali[4] demonstrated that normal pregnant women develop a refractoriness to the pressor effects of infused angiotensin II.[5] Angiotensinase, which inactivates angiotensin II, has been found to increase throughout the course of pregnancy[4] and may contribute to this refractoriness. Gant et al.,[3] Talledo et al.,[6] and Chinn and Dusterdieck[7] confirmed the increased sensitivity to infused angiotensin II in patients with or destined to develop the pregnancy-induced hypertension–preeclampsia syndrome. In normal pregnancy, no increased angiotensin-converting enzyme levels were noted by Parente et al.,[5] while Oats et al.[2] showed a steady increase in the angiotensin-converting enzyme level from 30 weeks' gestation until term. In a preliminary study, we demonstrated significantly increased angiotensin-converting enzyme levels in patients with pregnancy-induced hypertension when compared with those in normal pregnant women.

Since angiotensin-converting enzyme appears to have a pivotal role in the renin-angiotensin system by converting angiotensin I to angiotensin II, it seemed appropriate to undertake the present investigation—to study clinically the relationships of angiotensin-converting enzyme concentrations in pregnant patients who are normotensive and those with the pregnancy-induced hypertension–preeclampsia syndrome and patients with chronic hypertension with or without superimposed pregnancy-induced hypertension.

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Material and methods

Definitions

Pregnancy-induced hypertension. Pregnancy-induced hypertension was defined as an absolute blood pressure of 140/90 mm Hg or an increase in systolic blood pressure of 30 mm Hg and/or an increase in diastolic blood pressure of 15 mm Hg on two occasions, 6 hours apart, after 24 weeks' gestation.

Preeclampsia. Preeclampsia was defined as a triad of (1) the blood pressure changes noted in pregnancy-induced hypertension, (2) 24-hour urinary protein levels of ≥300 mg, and (3) generalized edema. For the purposes of this study, pregnancy-induced hypertension and preeclampsia were combined as components of a single abnormal physiologic and clinical syndrome.

Chronic hypertension. Chronic hypertension included the elevation in blood pressure noted for pregnancy-induced hypertension occurring either before pregnancy or before the twentieth week of gestation.

Superimposed pregnancy-induced hypertension. Superimposed pregnancy-induced hypertension was defined as clinical findings of pregnancy-induced hypertension in patients previously diagnosed as having chronic hypertension.

Magnesium sulfate. Magnesium sulfate was administered by the intravenous route with an initial bolus of 2 to 4 gm, followed by 2 gm/hr to maintain a serum magnesium level of 4.5 to 6.5 mg/dl. Patients were treated at the physician's desire in mild pregnancy-induced hypertension but routinely with severe pregnancy-induced hypertension–preeclampsia (blood pressure > 160/100 to 110 mm Hg). Notation was made of all patients receiving magnesium sulfate before angiotensin-converting enzyme levels were determined, and these were analyzed separately.

Mean arterial blood pressure. Mean arterial blood pressure was measured as:

$$\text{Diastolic blood pressure} + \frac{(\text{systolic blood pressure} - \text{diastolic blood pressure})}{2}$$

This was measured before 20 weeks' and after 24 weeks' gestation in patients with normal blood pressure, pregnancy-induced hypertension–preeclampsia, and chronic hypertension.

Patients. Pregnant women were selected at random from the obstetric population delivered of their infants at the Albany Medical Center Hospital from January 1, 1984, through July 1, 1985. The patients were studied in the third trimester of pregnancy on the basis of a clinical diagnosis of normal blood pressure, pregnancy-induced hypertension–preeclampsia, and chronic hypertension with or without pregnancy-induced hypertension (Table I). Samples of blood for measurement of serum angiotensin-converting enzyme levels were drawn either singly, about the time of delivery, or during antepartum evaluation. In addition, serum uric acid and blood urea nitrogen were determined. In 12 cases, nearly simultaneous samples for maternal venous and umbilical venous and arterial angiotensin-converting enzyme levels were obtained. Normotensive control patients were chosen at random either at the time of labor and delivery or elective cesarean section or antepartum in the third trimester of pregnancy. The 114 patients were drawn from a population of about 3325 women delivered of their infants during the study interval. The mean maternal ages were 25 ± 7 years in the normal population and 26 ± 7 years in the hypertensive population (p = NS). Racially, the groups were the same with 81% Caucasian, 16% black, and 3% Indian or Oriental. At the Albany Medical Center, approximately 15% of the deliveries are in patients who are transported in from a 17-county regional perinatal network. In the study, 30% of the patients represented maternal transports, since hypertensive disease is a major indication for transport. Both the normal and hypertensive patients had a mean gravidity of 2, and 48% were primigravid. Twenty-four percent of the patients smoked at least one half a pack of cigarettes a day—29% hypertensive women and 71% normal women. The mean weight gain in pregnancy was 33 pounds for both groups. The normotensive patients were delivered at a mean of 38 weeks' gestation, and the hypertensive patients at a mean of 36 weeks' gestation ($p < 0.025$). The babies delivered of normotensive women were heavier (mean 3015 gm) than those of hypertensive women (2570 gm) ($p < 0.02$), while the placental weights were not significantly different. There was no difference in the distribution of the sexes of the neonates. The hypertensive group tended to have a higher rate of cesarean delivery (55%) than the normotensive women (37%), but this was not statistically significant. Baseline control values of serum angiotensin-converting enzyme and uric acid were

Table I. Patient population: Distribution by diagnosis

Diagnosis		*n*
Normotensive		49
Pregnancy-induced hypertension–preeclampsia		57
No magnesium sulfate	36	
With magnesium sulfate	21	
Chronic hypertension		8
No pregnancy-induced hypertension–preeclampsia	5	
Superimposed pregnancy-induced hypertension–preeclampsia	3	
Total		114
Nonpregnant		9

There were five sets of twins in pregnancies with pregnancy-induced hypertension–preeclampsia and two in pregnancies without pregnancy-induced hypertension–preeclampsia.

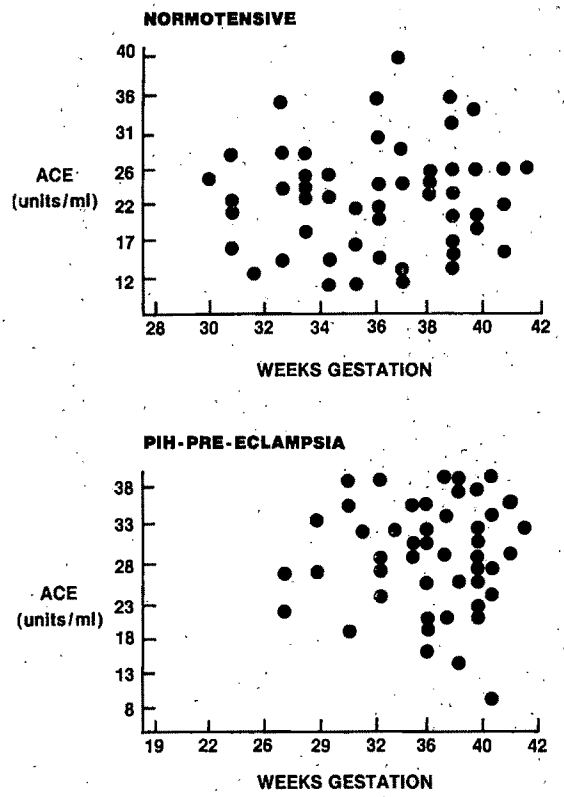


Fig. 1. Angiotensin-converting enzyme *(ACE)* levels in the normotensive and pregnancy-induced hypertension–preeclampsia populations compared by the week of gestation when samples were drawn [normal: $r^2 = 0.003$, $y = 25.5 + (-0.106x)$; pregnancy-induced hypertension: $r^2 = 0.027$, $y = 17.7 + 0.27x$].

measured in nine normotensive, nonpregnant, non–hormone-using women, aged 24 to 34.

Neonates. All babies were evaluated by the Neonatology Staff irrespective of the study. The neonatal complications were sepsis (two in normotensive mothers and two in hypertensive mothers) and respiratory distress syndrome (one in the normotensive group and nine in hypertensive women). One infant of a mother with pregnancy-induced hypertension died of congenital heart disease. The overall perinatal mortality was 8.7 per 1000 live births.

To correct for possible birth weight differences among babies born at differing gestational ages, all babies were classified as appropriate for gestational age, small for gestational age, and large for gestational age according to the University of Colorado data, which were used by the Albany Medical Center Neonatology Group. Mothers with pregnancy-induced hypertension were delivered of more small for gestational age babies than were those who were normotensive ($p < 0.025$) (normotensive: appropriate for gestational age, 44; small for gestational age, three; large for gestational age, four; hypertensive–pregnancy-induced hypertension: appropriate for gestational age, 35; small for gestational age, 14; large for gestational age, seven).

Laboratory. The angiotensin-converting enzyme levels were measured according to the method of Lieberman[8] in the Chemistry Laboratory of the Albany Medical Center Hospital. This method utilizes a spectrophotometric analysis of the free hippuric acid cleaved from hippuryl-L-histidyl-L-leucine by angiotensin-converting enzyme. The blood was drawn in a 10 ml clot tube, spun, and then kept either refrigerated or frozen at −4° C before analysis. The interassay variability is ±5 units/ml. The blood urea nitrogen and uric acid determinations were performed in the same laboratory.

Statistics. Data were analyzed by standard statistical procedures on the VAX computer with the use of mean, standard deviations, χ^2, and Student's t test.

Results

Angiotensin-converting enzyme levels (Figs. 1 and 2). The determinations of serum angiotensin-converting enzyme levels obtained from the twenty-fifth to the forty-third week of gestation from all patients with normal blood pressure and those with the pregnancy-induced hypertension–preeclampsia syndrome were examined graphically (Fig. 1) against the week of gestation during which the samples were obtained. The angiotensin-converting enzyme levels appeared to be independent of gestational age. Nonpregnant women had an angiotensin-converting enzyme level of 29.3 ± 6.4 units/ml, which was significantly greater than that of pregnant women with normal blood pressure (21.1 ± 6.9 units/ml, $p < 0.005$) (Fig. 2.). Among those women with the pregnancy-induced hypertension–preeclampsia syndrome, those receiving magnesium sulfate before the measurement of angiotensin-converting enzyme had levels of 22.6 ± 8.7 units/ml, which was lower than that of those with no magnesium sulfate therapy (29.1 ± 6.5 units/ml, $p < 0.005$). The angiotensin-converting enzyme level in women with chronic hypertension without pregnancy-induced hypertension (23.1 ± 2.7 units/ml) was less than that of those with superimposed pregnancy-induced hypertension (30.7 ± 4.4 units/ml, $p < 0.005$) and bore the same relationship as the patient with pregnancy-induced hypertension with or without magnesium sulfate therapy. Normotensive women had angiotensin-converting enzyme levels similar to those of patients with chronic hypertension and no pregnancy-induced hypertension and those with pregnancy-induced hypertension and magnesium sulfate therapy.

There were seven sets of twins; five had mothers with pregnancy-induced hypertension and two mothers were normotensive. When the angiotensin-converting enzyme levels were compared to those of the women

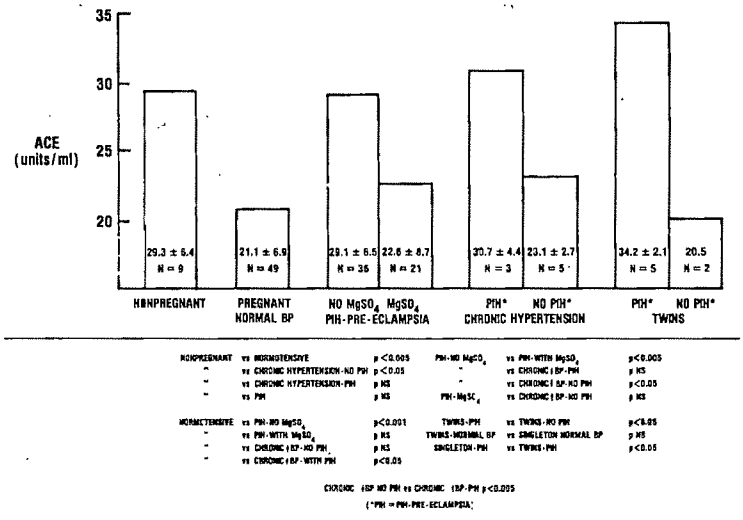


Fig. 2. Angiotensin-converting enzyme *(ACE)* levels in the various patient groups divided by the blood pressure status. *$MgSO_4$* = Treated with magnesium sulfate before angiotensin-converting enzyme determination. *No $MgSO_4$* = No magnesium sulfate given before angiotensin-converting enzyme determination.

Table II. Paired samples of maternal and cord blood angiotensin-converting enzyme levels, with samples obtained nearly simultaneously from each compartment

	Angiotensin-converting enzyme		
Diagnosis	*Maternal venous (units/ml)*	*Umbilical cord venous (units/ml)*	*Umbilical cord arterial (units/ml)*
Pregnancy-induced hypertension–preeclampsia, no magnesium sulfate	37.8	37.4	34.5
	34.0	27.2	
	37.8	37.8	
	37.8	47.7	
Normotensive	23.7	21.0	13.7
	22.0	37.0	22.0
	11.6	23.0	24.4
	21.0	10.2	
	19.0	22.2	18.8
	18.6	20.4	
Pregnancy-induced hypertension–preeclampsia with magnesium sulfate	19.7	21.7	15.7
Diabetes (Classes D to R), pregnancy-induced hypertension–preeclampsia	35.7	43.7	

With magnesium sulfate = Treated with magnesium sulfate before angiotensin-converting enzyme determination; no magnesium sulfate = no magnesium sulfate given before angiotensin-converting enzyme determinations. Results show: Maternal venous value was less than the cord venous value and greater than the cord arterial value.

with singleton pregnancies with or without pregnancy-induced hypertension, there were no differences.

The mothers whose babies were classified as small for gestational age (n = 17) had angiotensin-converting enzyme levels that were comparable to those of mothers of normal-weight babies from both the normotensive and hypertensive groups.

The study population had 10 patients with diabetes mellitus complicating pregnancy: (1) normotensive, Class A (n = 4) and Class D (n = 1); (2) pregnancy-induced hypertension, Class B (n = 2), Class C (n = 1), and Classes D to R (n = 1); (3) chronic hypertension, Class R (n = 1). There was a wide range of angiotensin-converting enzyme values in those patients, from 14.6 to 45.3 units/ml, which did not appear to correlate with either the diabetic classification or the normotensive-hypertensive category.

Paired samples, obtained nearly simultaneously, were drawn from the maternal venous and umbilical venous blood of 12 patients and umbilical arterial blood in six maternal/neonatal sets (Table II). The values, regardless of the status of blood pressure, showed a rather uniform relationship: The maternal venous value was less than the cord venous value and greater than the

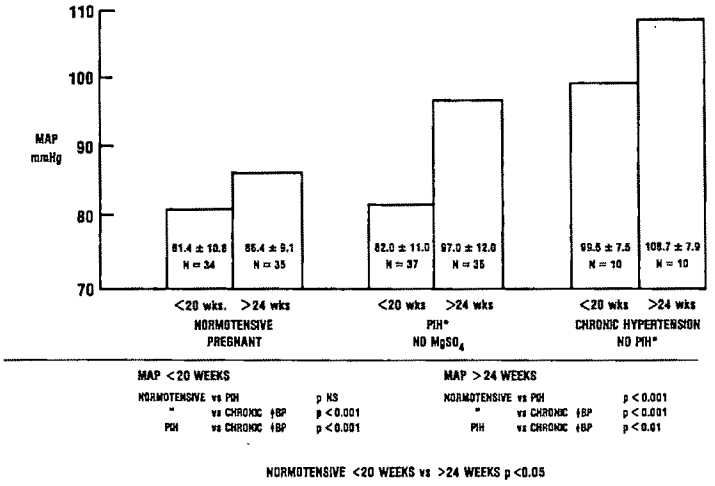


Fig. 3. Mean arterial blood pressure *(MAP)* in normotensive women, women with pregnancy-induced hypertension *(PIH)* in whom magnesium sulfate was not given, and patients with chronic hypertension before 20 weeks and after 24 weeks of gestation.

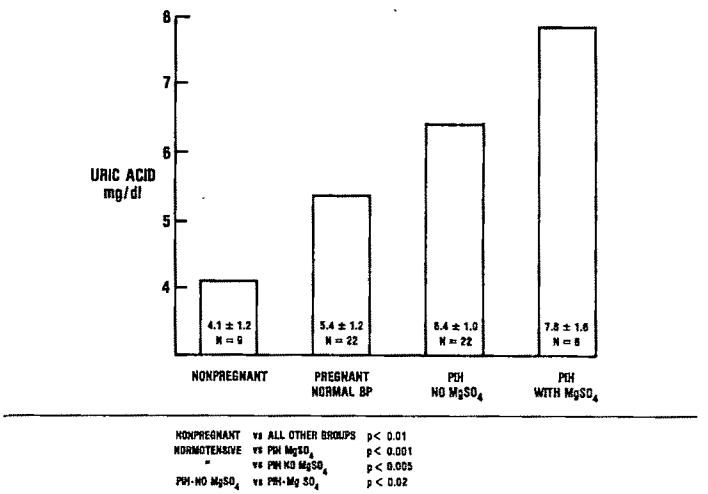


Fig. 4. Uric acid levels in patients according to the status of the blood pressure. $MgSO_4$ = Treated with magnesium sulfate before angiotensin-converting enzyme determination. *No* $MgSO_4$ = No magnesium sulfate given before angiotensin-converting enzyme determination.

cord arterial value. In four pregnancies, amniotic fluid was obtained for angiotensin-converting enzyme determination and was consistently <5 units/ml.

Mean arterial blood pressure (Fig. 3). The mean arterial blood pressure was calculated in normotensive women, women with pregnancy-induced hypertension–preeclampsia, and women with chronic hypertension before the twentieth week and after the twenty-fourth week of gestation. In each group, the mean arterial blood pressure at >24 weeks was significantly higher than that at <20 weeks (normal blood pressure, $p < 0.05$; pregnancy-induced hypertension, $p < 0.001$; chronic hypertension, $p < 0.001$). The mean arterial blood pressure at <20 weeks was the same in the normotensive group and the group with pregnancy-induced hypertension, and both were less than that from patients with chronic hypertension ($p < 0.01$).

Uric acid and blood urea nitrogen. The uric acid level (Fig. 4) in nonpregnant women (4.1 ± 1.2 mg/dl) was less than the third-trimester level in normotensive women (5.4 ± 1.2 mg/dl, $p < 0.01$). Patients with pregnancy-induced hypertension and not receiving magnesium sulfate had higher levels of uric acid (6.4 ± 1.0 mg/dl) than the normotensive women ($p < 0.005$), while those with pregnancy-induced hypertension and receiving magnesium sulfate had the highest values (7.8 ± 1.6 mg/dl, $p < 0.001$).

The blood urea nitrogen of normotensive women (9.2 ± 2.4 mg/dl) was less than that of patients with pregnancy-induced hypertension without (12.2 ± 3.9 mg/dl) or with (14.7 ± 6.8 mg/dl) magnesium sulfate

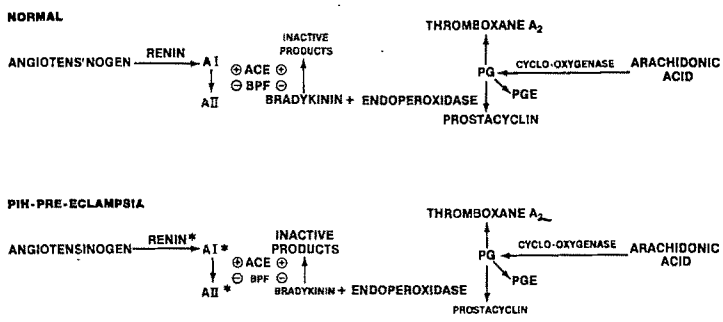


Fig. 5. Theoretical interrelationship of the renin-angiotensin system, bradykinin, and the prostaglandins in pregnant patients with normal blood pressure and those with the pregnancy-induced hypertension *(PIH)*–preeclampsia syndrome. * = Increased (?). *ACE* = Angiotensin-converting enzyme. *BPF* = Bradykinin-potentiating factor. + = Stimulates reaction. − = Slows or blocks reaction.

($p < 0.001$). However, in the two groups of patients with pregnancy-induced hypertension, there were no differences.

Comment

This study demonstrates that serum angiotensin-converting enzyme levels show significant changes in pregnancies that are complicated by hypertensive diseases when compared with pregnancies in normotensive women. We have shown that there is no correlation ($r = 0.05$) when the angiotensin-converting enzyme levels are compared to week of gestation (Fig. 1). This agrees with Parente et al.,[5] but other authors[2, 9] have described a steady increase in angiotensin-converting enzyme after 30 weeks of gestation. That our nonpregnant angiotensin-converting enzyme levels are considerably higher than those from normal pregnancy confirms the literature[2, 9]; these levels are comparable to those in patients with pregnancy-induced hypertension not requiring magnesium sulfate and those with chronic hypertension and with superimposed pregnancy-induced hypertension (Fig. 2). Abdul-Karim and Assali[4] found that the nonpregnant woman was more responsive than the normotensive pregnant woman to an infusion of angiotensin II. They stated that the beneficial effect of pregnancy on hypertension was related to elevated angiotensinase activity and an increased destruction of angiotensin II. The literature seems to favor decreased angiotensin II levels in patients with pregnancy-induced hypertension,[7] but Symonds et al.[10] reported increased angiotensin II levels in preeclampsia. They claim that the concentration of angiotensin-converting enzyme in pregnancy-induced hypertension is too high to be the rate-limiting step in angiotensin II production. Thus there is a dilemma as to whether there is increased angiotensin II production or increased angiotensin II inactivation. In either case, an elevated angiotensin-converting enzyme level could be expected to occur. Of importance is the loss of refractoriness of arterioles in pregnant patients who have or will have pregnancy-induced hypertension. Gant et al.[11] demonstrated that increased sensitivity to angiotensin II infusion was present by 18 weeks and was the earliest indicator of subsequent pregnancy-induced hypertension. Even changes in plasma volume did not significantly affect vascular sensitivity to angiotensin II. Gant et al.[12] correlated the positive pressor response in patients with pregnancy-induced hypertension in the supine position in the third trimester (rollover test) with the sensitivity to infused angiotensin II. This had a high predictive value for the future occurrence or absence of pregnancy-induced hypertension. Lee and Todd[13] showed increased angiotensin-converting enzyme and catecholamine levels in mothers with a positive rollover test.

The rise in angiotensin-converting enzyme levels from patients with pregnancy-induced hypertension appears at about the same time that the clinical disease becomes evident. Therefore, the angiotensin-converting enzyme levels did not seem to be able to predict which patients are at risk for pregnancy-induced hypertension. However, when a patient has elevated blood pressure in pregnancy, the angiotensin-converting enzyme value is able to identify those patients with a diagnosis of pregnancy-induced hypertension. To confirm that angiotensin-converting enzyme appears to be a late or acutely occurring phenomenon, one must look at pregnant patients with chronic hypertension. In this study, these women have angiotensin-converting enzyme levels comparable to those in normotensive women, until they develop superimposed pregnancy-induced hypertension. Then the angiotensin-converting enzyme levels become elevated as are those in normal patients who develop pregnancy-induced hypertension. This correlates with the findings of Gant et al.,[14] who showed the angiotensin II refractoriness to be similar in women with chronic hypertension and normotensive women until the superimposition of pregnancy-induced hypertension. Then angiotensin II refractoriness was lost. There was no difference in an-

giotensin-converting enzyme levels according to weeks of gestation in the third trimester. This would allow diagnostic impressions to be made irrespective of gestational age. An interesting and important observation is the effect of magnesium sulfate on angiotensin-converting enzyme levels in patients with pregnancy-induced hypertension. In patients receiving magnesium sulfate, the angiotensin-converting enzyme levels were decreased to those of normotensive women and those with chronic hypertension without pregnancy-induced hypertension. This phenomenon is probably not due to plasma volume expansion as the uric acid levels and blood urea nitrogen did not demonstrate a similar decrease. In fact, patients who receive magnesium sulfate and who have more severe pregnancy-induced hypertension have uric acid levels greater than those of women not receiving magnesium sulfate ($p < 0.02$) (Fig. 4). Is this then due to an effect of magnesium sulfate on the arteriolar endothelium where angiotensin-converting enzyme is produced, or is it due to interference with the angiotensin-converting enzyme assay? The answer to this is still unknown. Elevation of uric acid has come to be an indicator of the severity of pregnancy-induced hypertension, and this progressive elevation may be indicative of poor fetal outcome.

Twin pregnancies in patients with pregnancy-induced hypertension were associated with angiotensin-converting enzyme levels that were higher (34.2 ± 6.5 units/ml) than those of singleton pregnancies with pregnancy-induced hypertension (29.1 ± 6.5 units/ml, $p < 0.05$). However, with no pregnancy-induced hypertension, the angiotensin-converting enzyme (20.5 units/ml) was similar to that in normotensive women with singleton pregnancies (21.1 ± 6.9 units/ml, p NS). The explanation does not lie with the placental size, as the total placental weights were comparable in the twin gestations with or without pregnancy-induced hypertension.

From the literature, paired samples of maternal venous and umbilical venous and arterial angiotensin-converting enzyme levels have shown that the angiotensin-converting enzyme is the same in all three spaces, that the cord venous levels are greater than cord arterial or maternal venous levels, or that the umbilical venous angiotensin-converting enzyme concentration is less than that in maternal venous blood.[2] In our study (Table II), the values suggest that the maternal venous value is less than the cord venous value and greater than the cord arterial value. The question remains if these differences are real. An additional source of angiotensin-converting enzyme in pregnancy may well be the placenta,[15] with distribution to both maternal and fetal circulations. The amniotic fluid levels are significantly less than in the other compartments (<5 units/ml) and, therefore, are probably of little significance.

An increased angiotensin-converting enzyme concentration is not exclusive to pregnancy-induced hypertension. Angiotensin-converting enzyme is shown to be elevated in sarcoid, thyroid disease, and in neonates with respiratory distress syndrome, and we now have one patient with systemic lupus erythematosus at 8 weeks' gestation, with normal blood pressure, whose angiotensin-converting enzyme level is 34.8 units/ml. Lieberman and Sastre[16] reported elevated angiotensin-converting enzyme levels in diabetic patients, but our experience shows variable values that do not seem to be predictive of whether the patient has pregnancy-induced hypertension. Observation of mean arterial blood pressure (Fig. 3) before 20 and after 24 weeks in normotensive gestations demonstrated a rise in the third trimester of pregnancy. This may well correspond with the mild decrease and plateau in angiotensin II refractoriness shown by Gant et al.[3] The mean arterial blood pressure in patients with pregnancy-induced hypertension and chronic hypertension with or without superimposed pregnancy-induced hypertension followed expected patterns.

The theoretical interrelationships of the components that control blood pressure in normal pregnancy and those complicated by pregnancy-induced hypertension–preeclampsia are seen in Fig. 5. The renin-angiotensin system seems to be well established. Estrogen may drive the system by increasing angiotensinogen substrate, as pregnancy is associated with increased maternal-placental estrogen levels. Although angiotensin-converting enzyme is predominantly found in the lung[15] and is the usual site of conversion for angiotensin I to angiotensin II, in pregnancy, the placenta[15] may play an important and additional role in this system. Normally the increased substrate may lead to increased angiotensin II but progressive increases in angiotensinase[4] protect the mother from the angiotensin II. Bradykinin, a potent vasodilator, sits in balance with angiotensin II. It is a competitive inhibitor of angiotensin I conversion, and bradykinin-potentiating factors inhibit the destruction of bradykinin and block the conversion of angiotensin I to angiotensin II. However, bradykinin is inactivated in the lungs, which suggests that angiotensin-converting enzyme may well promote this inactivation of bradykinin or blocking of bradykinin-potentiating factors. An imbalance in this system may then explain the increased sensitivity of the arterioles to angiotensin II and the clinical presentation of pregnancy-induced hypertension. The linkage of this system to the prostaglandins was provided by the facts that indomethacin, a prostaglandin synthetase inhibitor, decreased the refractoriness to angiotensin II in normal pregnant women, that infusions of prostaglandin E_2 lower angiotensin II sensitivity, and that prostaglandin I_2 (prostacyclin) was responsible for va-

sodilation, decreased platelet adhesiveness, and prevention of fibrin deposition. Prostaglandin I_2 was found to be decreased in pregnancies associated with pregnancy-induced hypertension, leaving a relative imbalance with increased thromboxane A_2. Thromboxane A_2 is released from the platelets and is responsible for vasoconstriction, platelet adhesiveness, and fibrin deposition. Thus Page's "inner vicious circle"[1] of decreased uterine placental perfusion associated with vasoconstriction, damage to the placenta, thromboplastin release, intravascular coagulopathy, fibrin deposition in the kidney, decreased glomerular filtration rate, sodium and water retention, and intracellular shift of sodium to increase vascular sensitivity to pressors explains the clinical situation of pregnancy-induced hypertension. However, the critical piece in this puzzle is the finding that bradykinin has control over the production of prostaglandin E and prostaglandin I_2 and bridges the gap between the renin-angiotensin system and the prostaglandins in the physiologic control mechanisms of pregnancy-induced hypertension. Angiotensin-converting enzyme, by its role in conversion of angiotensin I to angiotensin II and the inactivation of bradykinin, may be partially responsible for the decrease in prostaglandin E and prostaglandin I_2 in pregnancy-induced hypertension–preeclampsia.

The current study has shown significant relationships of angiotensin-converting enzyme to the clinical pictures of pregnancy-induced hypertension that occur in previously normotensive and chronically hypertensive pregnant women. Simultaneous measurements of several of the components of the systems controlling vascular reactivity are required and may confirm the now theoretical mechanisms responsible for the maintenance of normal blood pressure in normal pregnancy or the occurrence of elevated blood pressure in pregnancy-induced hypertension–preeclampsia.

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We will furnish a complete list of references upon request.

Discussion

DR. NORMAN GANT, Dallas, Texas. I thoroughly enjoyed reading the paper by Goldkrand and Fuentes. The authors observed that the angiotensin-converting enzyme was elevated in patients with pregnancy-induced hypertension when compared with normotensive pregnant women or pregnant women with uncomplicated chronic hypertension. In fact, the serum levels of angiotensin-converting enzyme were elevated in patients with pregnancy-induced hypertension to values essentially the same as observed in nonpregnant subjects.

The authors also observed that the angiotensin-converting enzyme levels were reduced to normal levels in the third trimester of pregnancy in women with pregnancy-induced hypertension who were treated with magnesium sulfate. The authors then compared these serum angiotensin-converting enzyme values to mean arterial blood pressures as well as blood urea nitrogen and uric acid values obtained in the same patients. As one might expect, uric acid and blood urea nitrogen levels were elevated in patients with pregnancy-induced hypertension whether or not they were treated with magnesium sulfate.

The authors also simultaneously measured serum angiotensin-converting enzyme values in women and in the cord blood of their infants. Unfortunately, there were not enough values to draw any firm conclusions from this portion of the study nor were there enough

values obtained in patients with diabetes to draw any firm conclusions.

The authors discussed their observations and proposed an interesting hypothesis that angiotensin-converting enzyme might be involved in the suppression of bradykinin. This in turn might result in a decreased production of prostacyclin and prostaglandin E_2 in vascular endothelium resulting in the increased sensitivity to angiotensin II that precedes the onset of pregnancy-induced hypertension. I found this hypothesis fascinating and an idea that needs to be investigated. I have several questions, however, concerning the present study before I am willing to accept the role of angiotensin-converting enzyme in the proposed hypothesis.

The results reported for angiotensin-converting enzyme in the present study were reported as units per milliliter. Because these values were not reported as specific activities, it is entirely possible that what the authors are observing in this study is a constriction in plasma volume resulting in an artificial elevation of angiotensin-converting enzyme levels in women with pregnancy-induced hypertension. They mention this but do not elaborate on this possibility. I believe it is imperative that this issue be resolved.

The observation that magnesium sulfate lowers angiotensin-converting enzyme values to normal levels in patients with pregnancy-induced hypertension is interesting and may well be, as suggested by the authors, the result of magnesium sulfate acting directly on endothelium to increase the production of angiotensin-converting enzyme. Certainly there is a recent report that magnesium sulfate stimulates vasodilation and an increase in prostacyclin in the human umbilical cord (Altura et al., Science 1983;221:376). It also may be that magnesium sulfate has a similar affect by increasing the angiotensin-converting enzyme production as well as prostacyclin production in vascular endothelium. If so, this would be a valuable piece of information that would be yet another reason for using magnesium sulfate to arrest and prevent seizures in patients with pregnancy-induced hypertension as well as to increase tissue perfusion in the mother as well as the fetus.

The authors also acknowledge that magnesium sulfate might interfere with the assay of angiotensin-converting enzyme. It is a very simple matter to ascertain if this is a possibility by simply adding magnesium sulfate to serum obtained from untreated preeclamptic women and then running the assay. In this way one would have eliminated the endothelium as a possible source of angiotensin-converting enzyme and could in a very short period of time ascertain whether the magnesium sulfate is merely interfering with the assay.

In closing, let me say that the authors should be congratulated for a carefully conducted clinical study. Their review of the literature and their hypothesis for a mechanism to explain their observations were well done. I urge them to resolve, as soon as possible, the questions that I have raised concerning what may very well be an extremely important observation in our continuing search for a cause of pregnancy-induced hypertension.

DR. GOLDKRAND (Closing). First I would like to thank Dr. Gant for his kind remarks and for preparing for us the next stage in our investigation.

I would also like to take this opportunity to thank my coinvestigator, Dr. Fuentes, who was really one of the prime movers in this project and I think deserves credit for his work.

In relation to the comments that Dr. Gant made, I think they are very important as to whether we are seeing an effect that is due to constriction of blood volume. However, we have not yet looked at other factors. The suggestion of using Evans blue dye to evaluate the supposition that this may be a blood volume phenomenon raises a real question. The fact is that the change in angiotensin-converting enzyme does not occur early. It is a late-occurring phenomenon.

If the sample for measurement of angiotensin-converting enzyme is drawn early, it does not really have predictive value. Differential diagnosis is possible in the patient who presents with hypertension, but there may be an abnormality of the elevated enzyme. Dr. Gant's comments may be true and have yet to be elucidated.

The effect of magnesium sulfate is certainly one that needs to be evaluated very soon. We have also wondered about this effect. With our use of Swan-Ganz catheters in some of our extremely ill patients, we have not really seen a major change in some of the factors that might accompany the production of prostacyclin and block the potential action of angiotensin-converting enzyme.

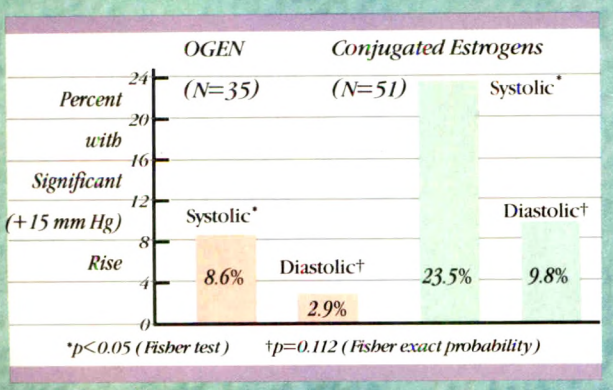
Part of the problem is that the true half-life of this enzyme is not known because it has not been well worked out, and the effects that may be taking place from a therapeutic modality may not immediately demonstrate any change. At other times, when using other treatment modalities for a longer time, we do see changes both in blood volume and in stabilization of patients, so there may be a time factor here that needs to be evaluated.

However, the effect of magnesium sulfate on the assay is certainly a question that is begged very strongly and is about to be answered in the laboratory by the addition of magnesium sulfate to the blood sample to see if it has a direct effect on the assay method.

Her estrogen therapy shouldn't affect her blood pressure



Percent of patients showing BP change of 15 mm Hg or greater after 7.4 months to 7.9 months therapy with either conjugated estrogens or OGEN® (estropipate)



Adapted from Wren et al, 1981.[1]

OGEN dosages ranged from 0.625 to 2.5 daily, 24 out of 30 days. Conjugated estrogen dosages ranged from 0.3 to 2.5, 24 out of 30 days. The majority of those experiencing a change, like the majority of the total sample, were also taking a progestogen, levonorgestrel 0.03 mg, from the 15th to the 24th day.

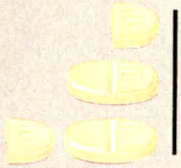
Because the risk of hypertension increases with age, it is appropriate to examine the effects of estrogen replacement on blood pressure.

"More women receiving...[conjugated estrogens] had a clinically significant rise in blood pressure than those receiving Ogen..."[1]

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

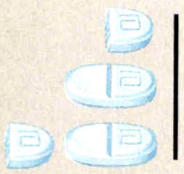
Scored tablets make precise dosing easy



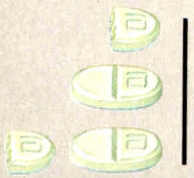
OGEN .625
(estropipate 0.75 mg, calculated as sodium estrone sulfate 0.625 mg)



OGEN 1.25
(estropipate 1.5 mg, calculated as sodium estrone sulfate 1.25 mg)



OGEN 2.5
(estropipate 3 mg, calculated as sodium estrone sulfate 2.5 mg)



OGEN 5
(estropipate 6 mg, calculated as sodium estrone sulfate 5 mg)

OGEN tablets are scored so you can adjust dosage without writing a new prescription. It's another reason more physicians are prescribing OGEN.

OGEN®
(estropipate)

Only estrone. Identical to the body's own.

See adjacent page for brief summary of prescribing information.

Reference:
1. Wren BG, Routledge DA: Blood pressure changes: Oestrogens in climacteric women. *Med J Aust* 2:528-531, 1981.

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Abbott Pharmaceuticals, Inc.
North Chicago, Illinois 60064

OGEN®

ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prucent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.
2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.
3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.
4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.
5. Preexisting uterine leiomyomata may increase in size during estrogen use.
6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.
7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.
8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.
9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of *broad spectrum antibiotics* which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving *insulin* may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Abnormal glucose tolerance test results.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
   Increase in size of uterine fibromyomata.
   Vaginal candidiasis.
   Cystitis-like syndrome.
   Dysmenorrhea.
   Amenorrhea during and after treatment.
   Change in cervical eversion and in degree of cervical secretion.
   Breakthrough bleeding, spotting, change in menstrual flow.
   Premenstrual-like syndrome.
2. *Breast.*
   Tenderness, enlargement, secretion.
3. *Gastrointestinal.*
   Cholestatic jaundice.
   Vomiting, nausea.
   Abdominal cramps, bloating.
4. *Skin.*
   Hemorrhagic eruption.
   Erythema nodosum.
   Erythema multiforme.
   Hirsutism.
   Chloasma or melasma which may persist when drug is discontinued.
   Loss of scalp hair.
5. *Eyes.*
   Steepening of corneal curvature.
   Intolerance to contact lenses.
6. *CNS.*
   Chorea.
   Mental depression.
   Migraine, dizziness, headache.
7. *Miscellaneous.*
   Aggravation of porphyria.
   Edema.
   Reduced carbohydrate tolerance.
   Increase or decrease in weight.
   Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms —One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. *Given cyclically:*

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure —A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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THE FOURTH LIPO-SUCTION WORKSHOP FOR GYNECOLOGISTS

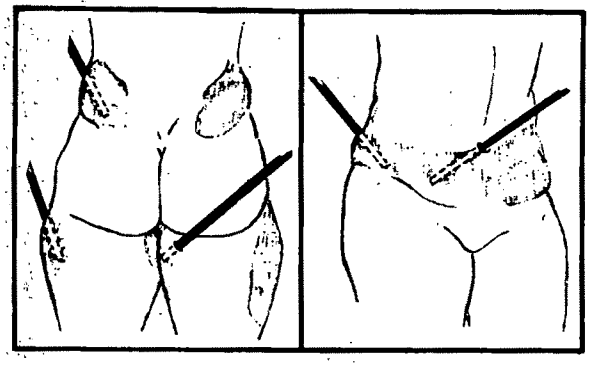
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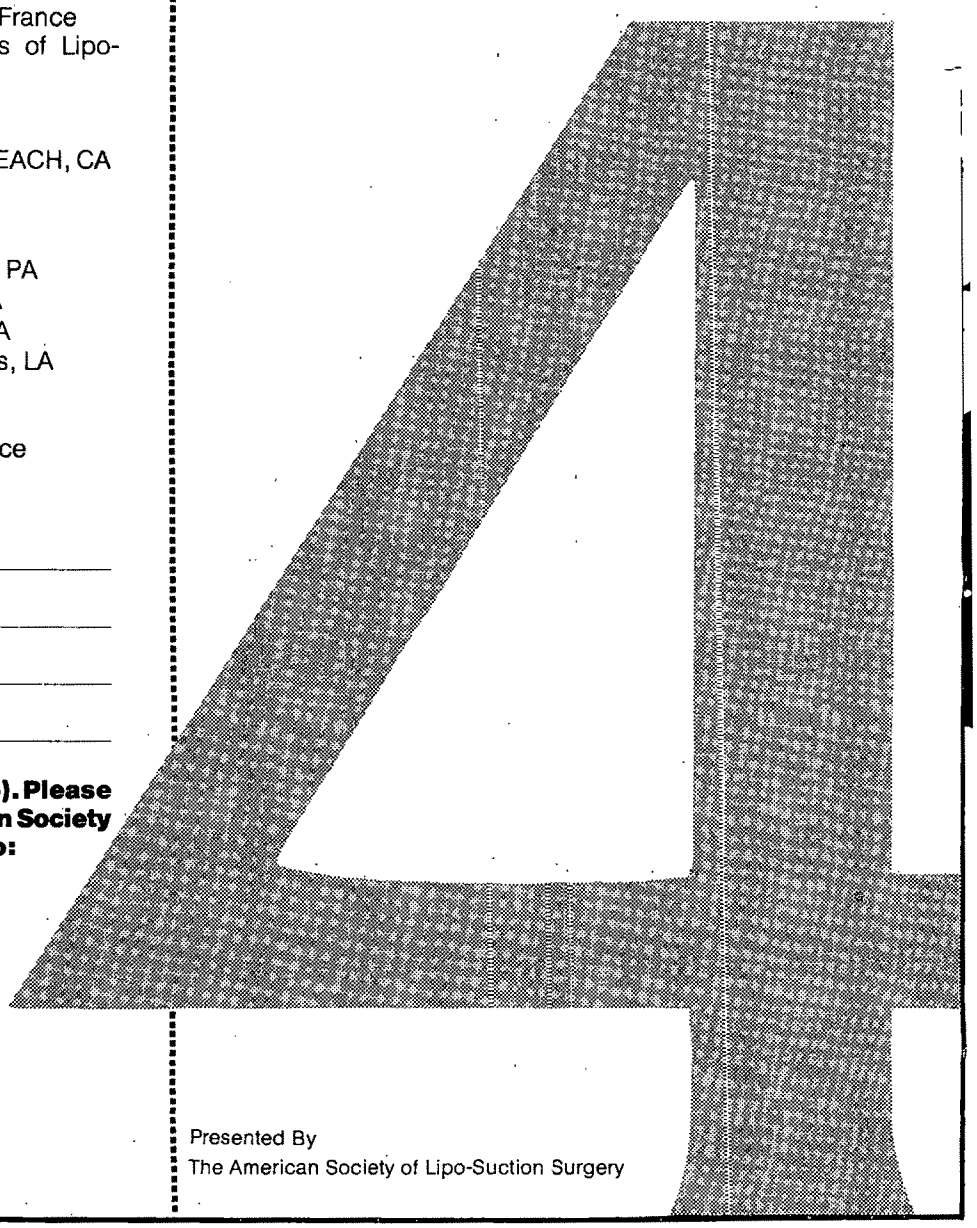
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PHONE ( ) _____

Course fee is $675 (Residents $475). Please make checks payable to the American Society of Lipo-Suction Surgery and mail to:
Frank Minyard, M.D.
Program Coordinator
921 Canal Street
New Orleans, LA 70112
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For additional information call: (504) 581-3520



Presented By
The American Society of Lipo-Suction Surgery

Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy

Mary Ellen Dorko, B.S., and T. Terry Hayashi, M.D.
Pittsburgh, Pennsylvania

Tritium-labeled ribonucleic acid precursors, including cytidine, uridine, and orotic acid, were injected into rats with dated pregnancies (14 to 21 days) and virgin rats. The acid-insoluble counts indicating incorporation into fetal and placental tissues showed that the highest incorporation occurred with cytidine, particularly earlier in pregnancy. In contrast, uridine demonstrated a minor degree of incorporation but displayed facile and enhanced transplacental passage with duration of pregnancy as represented by acid-soluble counts. Orotic acid was minimally used by both fetal and placental tissues. The incorporation of labeled precursors into maternal liver, heart, and kidney demonstrated varying responses during the course of pregnancy. (AM J OBSTET GYNECOL 1986;154:801-5.)

Key words: Tritium-labeled ribonucleic acid precursors, pregnant rats, fetal and placental ribonucleic acid incorporation

The incorporation of various ribonucleic acid (RNA) precursors into fetal and maternal tissues differs under various conditions.[1-4] There is enhanced incorporation of cytidine in comparison with uridine and orotic acid by the maternal rat liver,[5] and RNA metabolism in the maternal liver changes in accordance with fetal nutritional demands at various gestational ages.[5] Our previous studies demonstrated enhanced transplacental passage of uridine during late pregnancy.[1] The present study includes experiments with uridine, orotic acid, and cytidine during the last trimester of pregnancy in rats. The study was designed to assess transplacental passage and placental and fetal incorporation of cytidine, uridine, and orotic acid. We also took advantage of the labeled animals to study changes in RNA metabolism in maternal liver, heart, and kidney during pregnancy. In the current study the labeled precursors were administered intravenously rather than intraperitoneally as in previous reports.

Material and methods

Sprague-Dawley rats (Zivic-Miller, Allison Park, Pennsylvania) fed ad libitum included dated, pregnant rats and virgin rats. Labeled precursors were (5-^{3}H) cytidine (23 Ci/mmol), (5-^{3}H) uridine (20 Ci/mmol), and (5-^{3}H) orotic acid (21 Ci/mmol). Under light anesthesia with methoxyflurane, isotonic saline suspensions of labeled precursors (10 μCi/100 gm body weight) were injected into the saphenous vein with a beveled (25-gauge, ½-inch) needle. After 120 minutes, 3 ml of blood was removed by cardiac puncture, the animals were killed by cervical dislocation, and the various tissues were resected. The minimum number of rats in each group was five.

The 2-hour pulse labeling time was selected based on earlier studies indicating that the maximum incorporation of the three precursors had occurred during the first 2 hours after intravenous injection,[1] although orotic acid did continue to be incorporated slowly for hours.

Preparation and treatment of tissue extracts. The 3.0 ml cardiac tap blood sample was centrifuged at 10,000 × g for 15 minutes. A 0.1 ml aliquot of the serum was placed in a scintillation vial with 10.0 ml of 3a70B cocktail (Research Products International Corporation, Mt. Prospect, Illinois) and assayed for radioactivity.

The various tissues were minced, suspended in nine volumes of cold 0.1 mol/L sodium acetate buffer, pH 5.0, and homogenized with 10 strokes with a Potter-Elvehjem Teflon-pestle tissue grinder (Thomas Scientific, Swedesboro, New Jersey). A one tenth volume of 50% cold trichloroacetic acid was added to the homogenate, incubated on ice for 20 minutes, and centrifuged at 10,000 × g for 10 minutes. The resulting acid-soluble supernate was removed and the precipitate washed twice with cold 5% trichloroacetic acid. A 0.5 ml aliquot of the pooled supernate and washings was placed in 10.0 ml of 3a70B, and the acid-soluble radioactivity measured in a Packard Tri-Carb liquid scintillation system (Packard Instrument Co., Inc., Downers Grove, Illinois).

To the precipitate, 5.0 ml of 10% trichloroacetic acid was added and the mixture heated at 100° C for 15 min-

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Presented at the Fourth Annual Meeting of the American Gynecological and Obstetrical Society, Hot Springs, Virginia, September 4-7, 1985.
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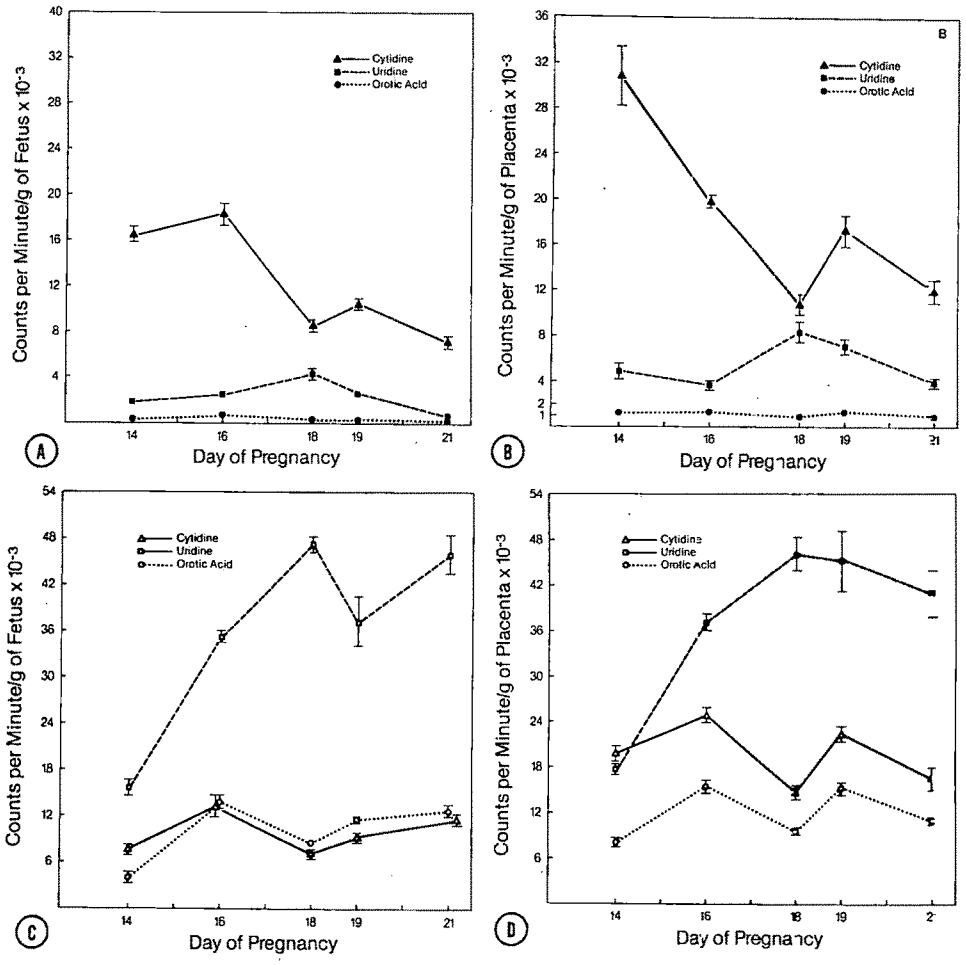


Fig. 1. Comparison of tritium-labeled cytidine, tritium-labeled uridine, and tritium-labeled orotic acid incorporation into fetal and placental tissues 2 hours after injection. RNA precursor radioactivity is measured in acid-insoluble counts (*A*, fetus; *B*, placenta) and acid-soluble counts (*C*, fetus; *D*, placenta). Each point represents five to 10 determinations (mean ± SEM).

utes. After cooling and centrifuging the sample, 0.5 ml aliquots of the acid-insoluble solution were assayed for radioactivity. All procedures were carried out at 4° C.

Results

The acid-insoluble counts per gram of tissue, which indicate the amount of precursors incorporated into the tissue, clearly showed that the greatest incorporation into both the fetal and placental tissues occurred with tritium-labeled cytidine (Fig. 1, *A* and *B*). The placenta showed approximately twice the amount of incorporation compared to the fetus. Although the total amount of radioactivity incorporated into both fetuses and placentas increased with the duration of pregnancy because of rapidly increased total weight and size, the greater amount of radioactivity per unit mass of tissue was measured in the earlier stages of pregnancy. The degree of incorporation with uridine and orotic acid was minimal. There was a marginal decrease in cytidine incorporation and slightly increased incorporation of uridine in the rats with pregnancies of 18 days.

The acid-soluble counts per gram of tissue, representing the amount of precursors taken up by tissue but not incorporated (Fig. 1, *C* and *D*), indicated enhanced placental and fetal transfer of uridine with increasing duration of pregnancy. Orotic acid showed minimal and consistent levels of placental and fetal transfer during pregnancy.

The incorporation of RNA precursors into maternal tissues varied with the organ involved (Fig. 2, *A*, *B*, and *C*). In the maternal liver (Fig. 2, *A*), both cytidine and orotic acid had depressed incorporation initially in early pregnancy but later increased to reach nonpregnant levels near term. There was minuscule incorporation of labeled uridine. The adult heart (Fig. 2, *B*) demonstrated the highest incorporation with cytidine but the degree of incorporation was decreased twofold during pregnancy. Uridine and orotic acid had minimal incorporation throughout the study. The maternal kid-

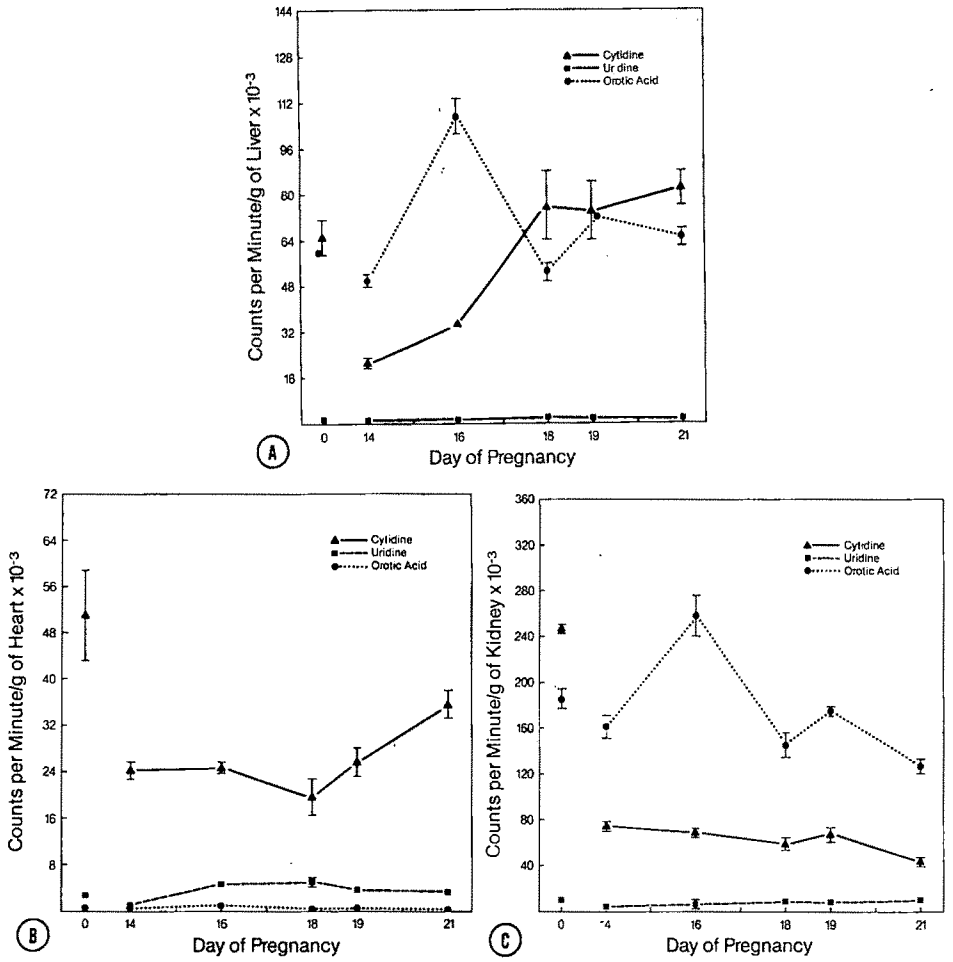


Fig. 2. Comparison of tritium-labeled cytidine, tritium-labeled uridine, and tritium-labeled orotic acid incorporation into maternal liver *(A)*, maternal heart *(B)*, and maternal kidney *(C)* 2 hours after injection. RNA precursor radioactivity measured in acid-insoluble counts, each point representing five to 10 determinations (mean ± SEM).

ney (Fig. 2, *C*) revealed the most unusual changes with pregnancy. In the nonpregnant state cytidine exhibited the highest incorporation, but during pregnancy orotic acid incorporation exceeded that of cytidine by twofold to threefold. However, as the duration of pregnancy increased, the incorporation of both orotic acid and cytidine declined. Again, there was only slight incorporation of uridine.

The cardiac tap serum consistently revealed the following radioactivity levels during pregnant and nonpregnant states: uridine, 54,000 to 90,000 cpm ml^{-1}; orotic acid, 21,000 to 26,000 cpm ml^{-1}; and cytidine, 11,000 to 14,000 cpm ml^{-1}.

Comment

The present study clearly indicates that of the three RNA precursors, cytidine is used most efficiently in the biosynthesis of placental and fetal RNA during the second half of pregnancy. This was somewhat unexpected since orotic acid is incorporated into RNA along the pathway of de novo synthesis while cytidine and uridine are incorporated along the salvage pathways. The enhanced incorporation of cytidine occurred in spite of facile placental transfer of uridine and raises the question of the absence of uridine kinase in fetal and placental tissue. Whether cytidine is used so effectively in the earlier stages of pregnancy remains to be examined. Such microsurgical studies are contemplated since early gestational experiments will have important implications for the study of teratogenesis and embryonic growth. It is interesting to note the marked mutagenic effect of a cytidine derivative in a microbial system.[7] The current gestational period that was studied, however, may provide appropriate background for the study of intrauterine growth retardation in a rat model being developed in this laboratory.

In a previous report Larkin and Stevens[6] demonstrated significant incorporation of tritium-labeled cytidine into both deoxyribonucleic acid (DNA) and RNA in 12-day pregnant rats especially in the junctional zone

of the placenta. The amount of DNA was not cited. Our future studies will include the use of carbon 14–labeled cytidine with alkaline hydrolysis used to determine the ratio of RNA/DNA labeling in both the fetus and placenta during the course of pregnancy. In addition, other studies will determine whether labeled cytidine is incorporated as such or as uridine.

The rapid and facile transfer of uridine to both fetus and placenta was surprising because the incorporation of uridine is so low. Additional short-pulse labeling experiments conducted at 15-minute intervals for 2 hours confirmed that uridine is indeed transferred rapidly and not used. There was no evidence of increased breakdown of uridine as suggested previously by Larkin and Stevens.[6] Future studies are planned to determine whether the bulk of acid-soluble uridine radioactivity remains as a nucleoside and is not phosphorylated. Uridine phosphorylase activity has been reported in adult rat blood.[8] In an earlier report we demonstrated the incorporation of ^{14}C-labeled uridine into fetal and placental uridine monophosphate in near-term pregnant rats.[1] The results noted in the 14-day pregnant rat (Fig. 1, *C* and *D*) suggest that the facile transfer of uridine may not extend to earlier stages of pregnancy.

In the nonpregnant state, both orotic acid and cytidine have been reported to be used much more than uridine for RNA synthesis in the rat liver.[9, 10] It is interesting that, in a recent report, Lewan et al.[11] demonstrated that in the male mouse the incorporation of tritium-labeled cytidine was two to three times greater than that of labeled uridine or orotic acid. Thus there are important species differences to consider as well as metabolic and hormonal influences. In our present study the incorporation of cytidine and orotic acid increased in the maternal liver during pregnancy to exceed nonpregnant values (Fig. 2, *A*). Simultaneously, the incorporation of cytidine decreased in both the fetus and the placenta. The changes may reflect either an increase in maternal liver RNA synthesis to provide nutrients for the enlarging fetoplacental unit or an enhanced ability of a mature fetoplacental unit to synthesize RNA precursors de novo, thereby decreasing the need for a large maternal supply.

The only significant incorporation in the maternal heart occurred with cytidine. The twofold decrease in cytidine incorporation with pregnancy may have resulted from the increased use of cytidine by the fetus and placenta or from increased circulatory demands in the maternal system. There was a modest increase in cytidine incorporation near term.

In the maternal kidney there was a striking threefold decrease in cytidine incorporation with pregnancy, which may have resulted entirely from the enhanced fetal and placental use. Orotic acid, which is not used in a similar fashion, demonstrated fairly constant incorporation in both pregnant and nonpregnant states. In contrast to the other two maternal organs, the incorporation of orotic acid and cytidine decreased near term, suggesting that the control of RNA synthesis in the kidney during pregnancy differs from that in the liver and heart.

The use of a beveled 25-gauge needle enabled us to inject the labeled precursors intravenously with consistency, thereby avoiding any accidental intra-amniotic injection, especially near term. There was no increase in the values of SD (not shown) and SEM of the measured radioactivity in the fetus and placenta in late pregnancy. Furthermore, the consistent values observed in the cardiac taps throughout the entire study, including pregnant and nonpregnant states, additionally reaffirmed the efficacy of the intravenous route. Our previous studies indicating fetal incorporation of uridine may have resulted from intra-amniotic injection of the isotope.[1] Greenman[12] has compared the use of subcutaneous and intraperitoneal injection of uridine in nonpregnant rats.

In summary, the present study demonstrated enhanced use of cytidine by the fetus and placenta, although there was a facile and rapid transfer of uridine to the fetoplacental unit. Studies performed with the maternal organs indicated that there are differences in the incorporation of RNA precursors among the various maternal organs during the different stages of pregnancy.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Sergio E. Fabro, Washington, D. C. The authors present data from experiments in which pregnant rats were treated intravenously with tritiated cytidine, uridine, or orotic acid at different stages of pregnancy. Two hours after dosing the animals were put to death and the tritium activity was measured in the acid-insoluble and acid-soluble fractions of homogenates of fetal and maternal tissues. After the administration of radioactive cytidine to rats at 14 days of pregnancy, the acid-insoluble fraction of fetal and placental samples contained significantly more radioactivity than the corresponding tissues in parallel experiments with radioactive uridine and orotic acid. Furthermore, whereas low levels of tritium incorporation into the acid-insoluble fraction of the fetus and placenta were consistently noted after uridine and orotic acid administration to rats at 16, 18, 19, and 20 days of pregnancy, the corresponding experiment with cytidine showed a decrease in tritium incorporation into the insoluble fraction that was approximately inversely related to the gestational age.

On the other hand, the acid-soluble counts showed similar results at different gestational ages for uridine and orotic acid. In the experiments with cytidine, however, the amount of radioactivity in the fetal and placental compartments increased as pregnancy progressed.

These experiments are the logical extension of earlier work by the same group, in which the relationship between maternal pharmacodynamics, placental permeability, and nucleic acid biosynthesis in the fetoplacental unit have been the subject of study; past contributions by this group have been significant in improving our understanding in these areas. This type of experiment, attempting to clarify placental permeability as well as fetal macromolecular synthesis at different gestational ages, is important since our knowledge in this area is meager at best. However, the data as presented today require additional information to be truly informative. In performing experiments with radioactive compounds, it is nowadays essential to identify the chemical entity that the radioactivity represents. As mentioned in the Comment section, the authors plan to carry out future experiments that will lead to the identification of the radioactivity. Thus a first step for understanding the significance of the amount of radioactivity found in fetal and placental tissues at different gestational ages would be the chemical identification of such radioactivity. In this regard, if one wants to be picky, he or she would ask an important initial question: How did the authors check the purity and radiostability of the tritium in the compounds used?

In terms of pharmacokinetics, why did the authors select 2 hours after treatment as a one-time examination for all three chemicals at different stages of pregnancy? The other difficulty I have in dealing with the data presented is related to the pool size of the radioactive chemicals in the specific maternal, fetal, and placental compartments. Thus the data as presented today can be interpreted only on the assumption that the pool sizes of the administered chemicals are equal and remain constant at different stages of pregnancy. This is an assumption that may not be correct.

In summary, I think it would add significantly to the scientific value of the study if the authors would identify the radioactivity and determine the actual pool size of the different precursors that were used in the various fetoplacental compartments at different gestational ages. It is a lot of work, but it will increase the scientific value of these data considerably.

Dr. Hayashi (Closing). I thought somebody was going to ask me the question: Why are you doing this experiment? Actually, we have developed a rat animal model for intrauterine growth retardation, and I wanted to find out the fetoplacental metabolism of that model. That is the reason we chose this approach.

Dr. Fabro's first question regarding the biochemical identification is critical for our future study, especially with the uridine radioactivity being so high. If I had to guess, the radioactivity represents uracil, which would explain everything because otherwise we are dealing with a brand new eukaryotype of uridine kinase.

Two hours was chosen, Dr. Fabro, because our previous studies during the course of pregnancy showed that maximum incorporation took place within 2 hours. I appreciate that for pharmacokinetics we should do more extensive studies.

In one of our previous studies we did measure pool size. I have one problem with just measuring the RNA pool size, because in a report by Larkin and Stevens (see reference 6) they have reported the incorporation of cytidine into DNA. They did not specify quantity, but they observed DNA especially in the junctional zone of the placenta. Thus in the early part of pregnancy, we may be seeing a higher DNA ratio, so alkaline hydrolysis may have to be performed to measure the DNA/RNA ratio. Furthermore, we may have to do phenolextraction of RNA to be sure about the exact RNA pool size. We have raised additional questions with the present study.

Uterine artery Doppler velocimetry in pregnant women with hypertension

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Stony Brook, New York

Uterine and umbilical artery velocimetry was carried out on 71 women with hypertensive disorders in pregnancy. Three categories of hypertensive disease were diagnosed: chronic hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia. Clinical classifications describe the severity of disease effectively, primarily because the classification is based on the appearance of abnormal physical or laboratory findings. Doppler velocimetry of the uterine arteries shows that normal pregnancy occurs when the systolic/diastolic ratio is ≤2.6. When the ratio exceeds this level and there is a notch in the waveform, the pregnancy is complicated by stillbirth, premature birth, intrauterine growth retardation, and maternal preeclampsia. The positive and negative predictive value of the examination is 93% and 91%, respectively. It appears that this new technology will be an essential ingredient of optimum pregnancy surveillance. (AM J OBSTET GYNECOL 1986;154:806-13.)

Key words: Doppler, ultrasound, uterine arteries, hypertension, pregnancy

The diagnosis and management of hypertension in pregnancy have been handicapped by the absence of a precise test that will separate chronic hypertension from preeclampsia.[1,2] A combination of historical, clinical, and laboratory factors are used to make a diagnosis and to formulate a plan of management.[3-5] Since a precise diagnosis is not possible, clinical decisions to induce labor or carry out a cesarean section are based on probability estimates and therefore are subject to error. It is also recognized that the majority of women with chronic hypertension will not develop superimposed preeclampsia, but an extensive surveillance process is recommended because of our diagnostic deficiencies. Concepts such as calculation of the mean arterial pressure, the "rollover test," and the angiotension infusion test have been introduced as an effort to identify early disease and develop treatment plans.[6,7] However, the sensitivity of these tests has been too low to attract widespread clinical usage.

Doppler arterial velocimetry provides a new noninvasive approach to the study and evaluation of the umbilical and uterine arteries.[8-11] In previous reports we described the value of umbilical velocimetry in normal fetal growth, intrauterine growth retardation, and discordant twin pregnancies. In this report we will present data that will demonstrate the power of this new technology in accurately identifying the status of the uteroplacental circulation in the presence of maternal hypertension. It will be shown that the presence or absence of the development of uterine artery compliance has a significant impact on the perinatal outcome and the degree of maternal systemic disease.

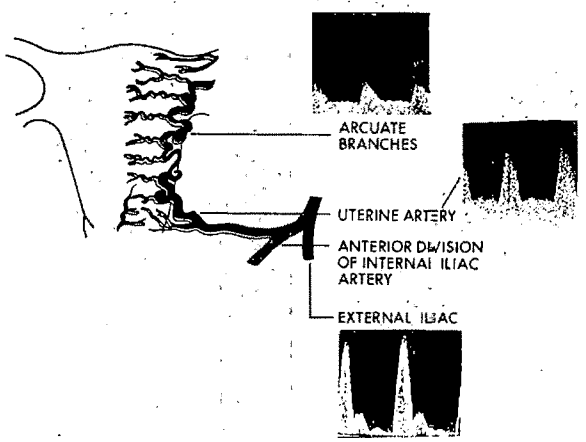


Fig. 1. Velocity waveform patterns of pelvic vessels at the twentieth week of pregnancy.

Material and methods

The patient population consisted of 71 pregnant hypertensive women whose cases were followed and who were delivered at Bronx Municipal Hospital Center and

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Table I. Clinical classification of laboratory and clinical parameters (mean ± SD)

	Chronic hypertension (n = 41)	*p*	*Chronic hypertension and preeclampsia (n = 14)*	*p*	*Preeclampsia (n = 16)*
Age (yr)	29.8 ± 7.6	NS	30.9 ± 7	<0.05	22.3 ± 5.6*
Parity (% para 0)	39	NS	31	<0.001	100†
Mean arterial pressure (mm Hg)	106 ± 8.3	<0.001	125 ± 13		121 ± 12†
Uric acid (mg/ml)	4.6 ± 0.1	<0.001	6.5 ± 1.7		7.2 ± 1.2†
Creatinine clearance (ml/min)	122 ± 31	<0.01	88 ± 33		76 ± 23†
Nonstress test (% positive)	10	<0.05	40		50*
Umbilical systolic/diastolic ratio	3 ± 0.8	NS	3.7 ± 1		4.2 ± 1.1*
Gestational age at delivery (wk)	39 ± 1.2	<0.001	33 ± 3		33 ± 3.3‡
Cesarean section for fetal distress (%)	18	<0.001	82		40*
1 min Apgar score of <7 (%)	15	<0.05	50	NS	36
Birth weight	3166 ± 514	<0.001	2069 ± 810	NS	1591 ± 568‡
Stillbirth (%)	0	NS	10	NS	20*
Uterine systolic/diastolic ratio	2.1 ± 0.4	<0.001	4.7 ± 2	NS	4.3 ± 1.9‡

*Difference from chronic hypertensive group of $p < 0.05$.
†Difference from chronic hypertensive group of $p < 0.01$.
‡Difference from chronic hypertensive group of $p < 0.001$.

Winthrop-University Hospital (formerly Nassau Hospital) during 1982 to 1984. The project was approved by the Institutional Committee on Human Investigation, and informed consent was obtained. The equipment used to obtain the arterial velocity waveform was a continuous-wave Doppler with a 4 MHz transducer and a power density of 6.5 mW/cm^2. A description of the equipment and the methodology has been published previously.[8] An attempt was made to obtain both umbilical and uterine velocity waveforms in each patient admitted to the study. The umbilical waveforms were obtained transabdominally with the patient in the left or right lateral tilt supine position. The systolic/diastolic ratio was used to quantitate these waveforms.

The uterine velocity waveforms were obtained vaginally or abdominally. In the early phase of these studies we thought that studies carried out through the vagina would provide easier access and more reliable uterine artery signals. This proved not to be generally true, and the vaginal approach was more uncomfortable. For the vaginal studies the transducer is placed into a sterile glove with coupling jelly and then introduced into the lateral vaginal fornix along the paracervical area. The pregnant uterine artery waveform has a specific contour and can be easily differentiated from the external iliac arteries (Fig. 1).

The abdominal studies were carried out by placing the transducer on the anterior abdominal wall in the right and left lower quadrants aimed into the lower uterine segment and gently moved around until the appropriate velocity waveforms were seen. A total of 59 women had vaginal studies, and 12 women had the waveforms obtained transabdominally. Our data on normal pregnant women have shown that the vaginal and abdominal systolic/diastolic ratios were identical after 26 weeks. Calculations represent the average value between the right and left uterine artery.

At the end of pregnancy these 71 women were then grouped into three categories based on clinical criteria, that is, chronic hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia. Patients with preexistent hypertension or second-trimester mean arterial pressures of 90 mm Hg were identified as being chronically hypertensive.[12] Those with an increase in blood pressure during the third trimester, and the presence of proteinuria and edema, in the absence of a history of hypertension were diagnosed as having preeclampsia. The third group represents women with chronic hypertension who experienced a significant increase in blood pressure with proteinuria, edema, or subjective symptoms that characterize preeclampsia.

In the chronic hypertensive group serial measurements from 16 weeks onward were obtained in 71% of the women and single measurements during the third trimester in the remaining 29%. In the other two groups this ratio is reversed, with 21% and 37% for serial measurements and 79% and 63%, respectively, for single measurements. For those with serial measurements, the third trimester average was used for the purpose of analysis.

Clinical management of chronic hypertension included antepartum nonstress tests, ultrasound examination, renal function studies, and fetal movement counting. Delivery before term was undertaken when there was fetal distress or significant maternal deterioration. Patients with preeclampsia were placed on magnesium sulfate on admission to the hospital. If the clinical picture improved in terms of blood pressure, urine output, lack of subjective complaints, and bio-

Table II. Outcome related to uterine systolic/diastolic ratios

	≤2.6 (n = 43)	>2.6 (n = 28)	p
Mean arterial pressure (mm Hg)	110.2 ± 9.1	115.3 ± 13	NS
Uric acid (mg/ml)	4.7 ± 0.7	7.1 ± 1.8	<0.001
Creatinine clearance (ml/min)	114 ± 32	87 ± 33	<0.01
Nonstress test (% positive)	6.8	54.1	<0.001
Umbilical systolic/diastolic ratio	2.4 ± 0.6	4.1 ± 0.8	<0.001
Gestational age at delivery (wk)	38.5 ± 2.2	33.2 ± 7	<0.001
Cesarean section for fetal distress (%)	29	50	<0.01
1 min Apgar score <7 (%)	21	38	NS
Birth weight (gm)	3044 ± 620	1919 ± 830	<0.001
Stillbirth (%)	0	17	<0.05
Small for gestational age (%)	7	59	<0.001

Table III. Predictive value and efficiency of tests for hypertension and pregnancy outcome

	Predictive value (%)			
	Positive test	*Negative test*	*Sensitivity (%)*	*Specificity (%)*
Mean arterial pressure (mm Hg)	71	66	62	70
Creatinine clearance (ml/min)	73	61	67	61
Uric acid (mg/ml)	73	61	62	70
Uterine arterial systolic/diastolic ratio of >2.6	86	86*	81*	90*
Uterine arterial notch	93*	91*	87*	95*

*$p < 0.05$ or 0.01 against other categories.

chemical data (normal liver function, coagulation profile, etc.) they were weaned off the magnesium sulfate and transferred to the antepartum ward. The velocity data were not used for clinical management.

Statistical methods included calculations of means, standard deviations, standard error of a percentage, standard errors of difference between means, Student's *t* test, and the predictive values and efficiency of medical diagnoses.

Results

Table I displays a summary of some clinical and laboratory parameters for the women and newborn infants in this study. The group characterized as having chronic hypertension were those who demonstrated some elevation of blood pressure but few other problems. Their data do not differ significantly from a normal population, although there was a higher than average number of positive nonstress tests and cesarean sections. We used the chronic hypertensive group as statistical control subjects against both other groups. It can be seen that the clinical classification works very well in separating benign disease from more serious problems. The chronic hypertensive group with superimposed preeclampsia and those with preeclampsia alone differed in only a few parameters. One of these differences was in the number of cesarean sections, but this is probably a statistical aberration. The pure preeclamptic groups were younger and also all were nulliparous. The umbilical artery systolic/diastolic ratios were higher in both the preeclamptic groups but were only significant for the purely preeclamptic group. This may have occurred because the values for the chronic hypertensive groups were also at the lower limit of abnormal.

Table II divides the study group into normal and abnormal uterine artery systolic/diastolic ratios. Normal uterine artery values were obtained from the study of 60 normal pregnancies. In the last 14 weeks of pregnancy the uterine systolic/diastolic ratio is 2 ± 0.3. The velocity wave incisura, or notch, disappears by the twenty-sixth week of pregnancy. It can be seen that the uterine systolic/diastolic ratio clearly divided the patients into normal and abnormal groups. The two parameters that were not different were the average blood pressure and the percentage of low 1-minute Apgar scores. It should be noted also that most of the calculated differences were at a statistical level of <0.001, which indicates the strength of this new test.

The efficiency of a medical test and diagnosis is measured by the sensitivity and specificity as well as the predictive value of a positive or negative test. To carry out this calculation we selected the parameters of an optimum perinatal outcome, delivery at 37 weeks or beyond or a fetus weighing ≥2500 gm. These data are displayed in Table III.

It can be seen that calculations from the uterine velocity wave are more sensitive, specific, and predictive than the blood pressure, creatinine clearance, or uric acid levels. We did not have sufficient 24-hour protein

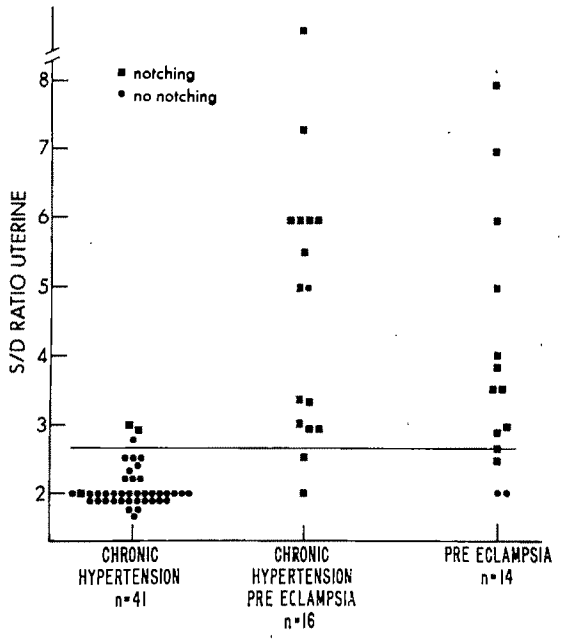


Fig. 2. Distribution of uterine systolic/diastolic *(S/D)* ratios within clinical classification of disorders caused by pregnancy hypertension.

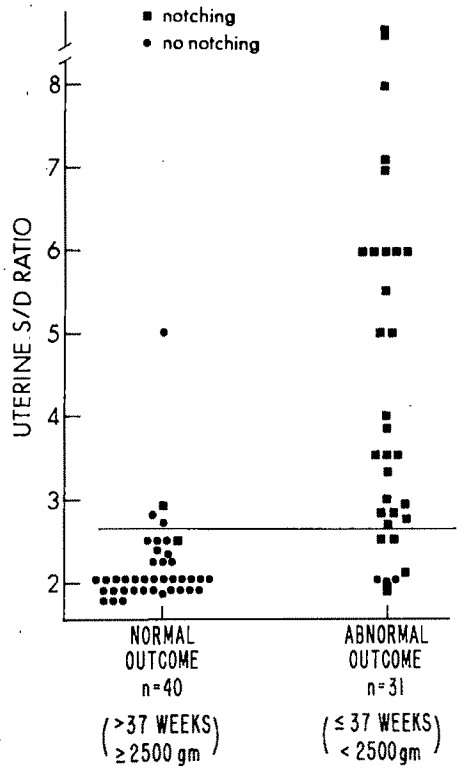


Fig. 3. Clinical outcome based on uterine systolic/diastolic ratios.

measurements to make similar estimates on this parameter. It is also interesting to note that a persistent notch in the uterine velocity wave is a more discriminating finding than measurement of the ratio alone. The presence of a notch is statistically significant against the other traditional measurements.

Fig. 2 provides distribution columns of ratios and notching within the designated clinical classifications. Again it can be seen that an elevated ratio or the presence of a notch provides virtual assurance of the presence of preeclampsia. Fig. 3 plots distribution columns for perinatal outcome as related to the uterine systolic/diastolic ratio and notching of the velocity waveform. With only a few exceptions a normal ratio was associated with a normal outcome. It can be noted that three of four women with normal ratios but having a persistent notch had abnormal outcomes, specifically, growth-retarded babies. In all three cases there were elevated umbilical artery systolic/diastolic ratios indicating significant placental disease. The one elevated ratio of 5 in the normal outcome group occurred as a single measurement late in the pregnancy. At that time the patient developed headaches, blurred vision, right upper quadrant pain, abnormal liver function tests, and was delivered of a 3985 gm baby. In all probability this was a gestational diabetic patient because the umbilical artery ratio was also elevated. In three other cases there were low birth–weight babies with normal uterine velocity waveforms. These women were delivered early because of the belief that the clinical situation was worsening. In one of these cases there was a probable error in diagnosis, since the woman continued to have hypertension and proteinuria months after the delivery. The other two cases had elevated umbilical artery systolic/diastolic ratios.

Fig. 4 displays the distribution of the perinatal outcomes of a normal and abnormal ratio and the percentage of low birth–weight babies and those who were growth retarded. It is clear that an abnormal ratio is associated with both premature delivery and intrauterine growth retardation.

Figs. 5 to 8 illustrate the patterns of development of uterine artery compliance in relation to the presence or absence of clinical disease. In normal pregnancy the uterine artery notching usually disappears by the twenty-sixth week and a normal ratio of 2 is seen from 28 weeks onward. As can be seen from these examples, several variations on the evolution of compliance can occur. Second-trimester measurements in the preeclamptic groups were not sufficient to know whether compliance was lost or never acquired.

Comment

Uterine and umbilical artery velocimetry provides information that is uniquely informative in the management of normal and abnormal pregnancies. In this paper, attention was directed to the role of these measurements in women suspected of being at risk for hypertensive complications of pregnancy. The abnormal

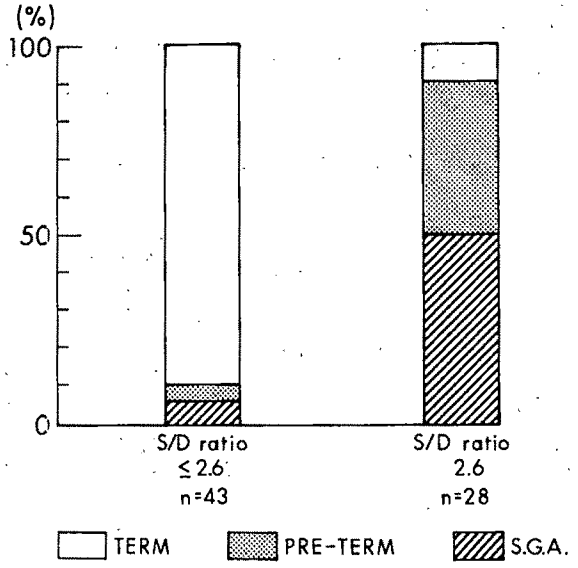


Fig. 4. Uterine vascular compliance and perinatal outcome. Data from Fig. 3 are displayed in this bar graph to show percentage of babies which were growth retarded and those who were average for gestational age but were delivered preterm.

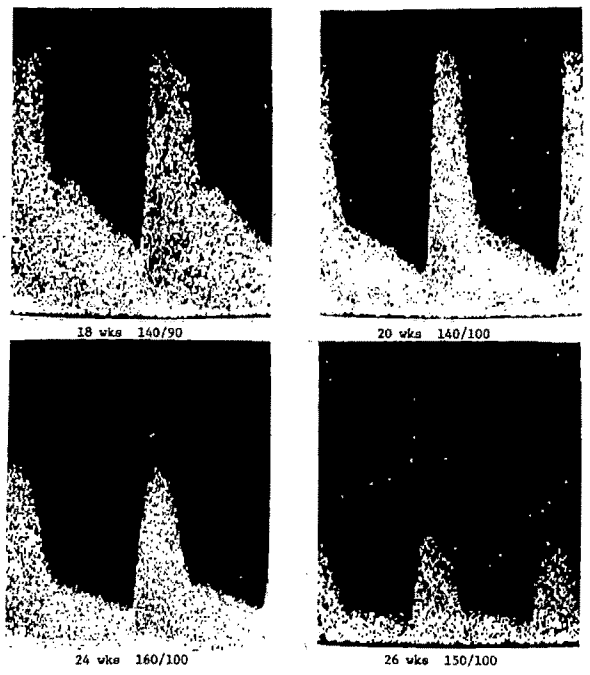


Fig. 5. Velocity waveform patterns of a woman with chronic hypertension who developed a normal vessel pattern by 24 weeks.

uterine artery velocity waveform has two easily measurable indices: the presence or absence of a notch in early diastole, and the peak-systolic/end-diastolic ratio.[11] If the ratio is elevated and a notch is present, the pregnancy suffers the consequences of inadequate nutrition and oxygenation.

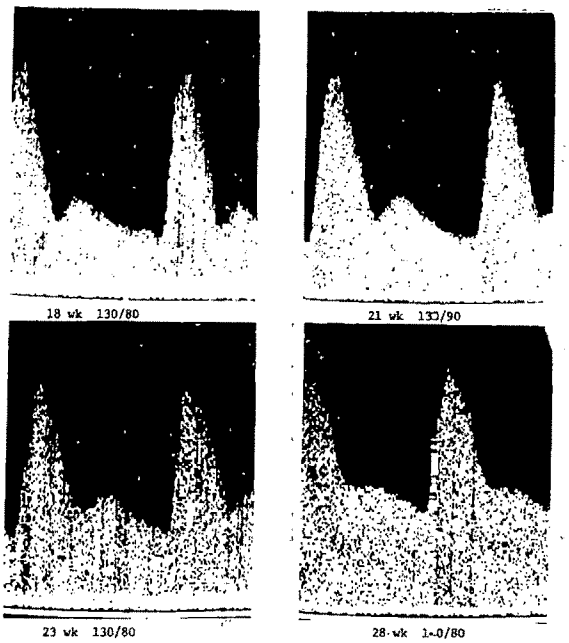


Fig. 6. Velocity waveform patterns of a woman with chronic hypertension in whom the normal vessel pattern did not develop until week 28.

To validate the usefulness of these measurements we analyzed their effect on perinatal outcome and the most common physical and chemical abnormalities seen in hypertension and pregnancy. The results revealed predictive values of ≥90% for both positive and negative tests. Thus for the first time the clinician has a tool that accurately describes the uteroplacental and fetoplacental circulation. Although velocimetry is only a component of flow, these studies demonstrate that for clinical circumstances it separates normal from abnormal. Of special interest is that the changes in uterine and umbilical velocimetry precedes by weeks or months the clinical manifestations of disease[13, 14] (Figs 6 to 8).

Campbell et al.[11] studied the uteroplacental circulation with use of a pulsed Doppler system coupled to a linear-array imaging of the branches of the uterine at the placental site. They created a calculation that was called the "frequency index profile." If a ratio calculation was applied to their derived waveform, the results were comparable to ours. When the normal waveform group was compared to the abnormal group, significant differences in outcome were recorded. These differences were not as large as those demonstrated in our study, probably because their patients were all seen at 35 weeks or later.[11]

Trudinger et al.[15] used the systolic/diastolic ratio for the analysis of uterine and umbilical velocity waveforms. They established a 95% value of approximately 1.8 for upper limits of normal with a mean of 1.5. They studied subplacental vessels only and the analysis was for the identification of infants who were small for ges-

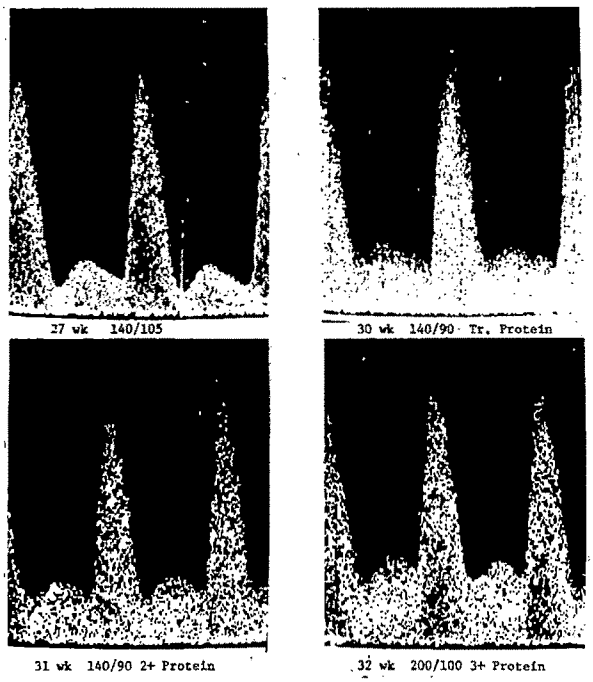


Fig. 7. Velocity waveform patterns of a woman with chronic hypertension who never did achieve a normal vessel pattern. She developed severe preeclampsia at 32 weeks.

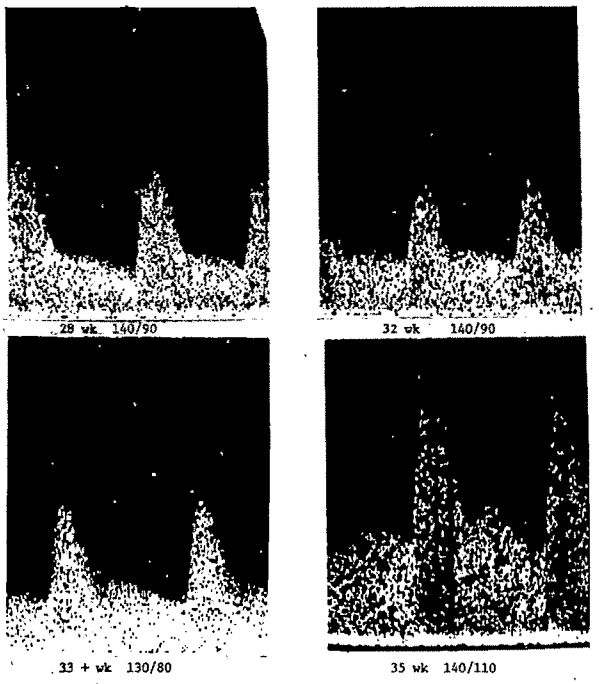


Fig. 8. Velocity waveform patterns of a woman with chronic hypertension who developed a normal vessel pattern and then reverted at 33 weeks. Preeclampsia was evident at 35 weeks.

tational age; therefore, their study is not comparable. As in our work, they also used a continuous-wave Doppler system, which is superior to a pulsed-wave system because of lower power density and lower expense.[15]

DeWolf et al.[16] have carried out systematic studies of the normal and abnormal vascular anatomy of the placental bed. In normal pregnancy trophoblastic erosion occurs in the spiral arteries during the process of placentation. In preeclamptic pregnancy "the myometrial segments of the placental bed spiral arteries are not invaded by cytotrophoblastic cells and do not undergo physiologic changes. They retain their musculoelastic arterial structure and, with the onset of the clinical signs and symptoms of preeclampsia, they are prone to develop hypertensive lesions."[16] The velocity waveform data complement these morphologic observations nicely in that the development of preeclampsia in most cases is associated with the failure of the development of normal low-resistance velocity flow in the uterine arteries.

The implications of the data recorded in this study are numerous. Hypertension is the most common medical complication of pregnancy. The blood pressure is a manifestation of a heterogenous group of disorders which may or may not impact on the pregnancy. Thus the ability to assess the functional state of the uterine and umbilical circulation provides the physician with an incisive probe that forecasts pregnancy outcome. This information provides a matrix on which treatment protocols can be devised. Although this represents another testing procedure during pregnancy, it should in the long run represent a net saving because the need for most nonstress tests and real-time ultrasound will be eliminated.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Luis A. Cibils, Chicago, Illinois. This new approach in the series of papers coming from Dr. Schulman's department focuses on the blood supply to the intervillous space. In my discussion of his study of the umbilical arteries presented to this Society 2 years ago I elaborated on some crucial technical points and posed some questions that were never answered to my satisfactions.[1] I am glad to have been given the chance to review the application of the same technique and principles to the assessment of uterine arteries flow and thereby restate some of my points with the hope that this time I will be given the clarifications I requested.

The most basic questions were posed by Kurjak and Rajhvajn[2] in 1982 when they stated that "ultrasonic studies should be tested in conjunction with electromagnetic flowmeter measurements, but this can be done only in animal experiments." Up to the present time this has not been done, and neither the more refined technique of range-gated–pulsed method nor the much more crude method of "visual pattern recognition" used in the present study has been clearly proved to be accurate. In addition to the problem of accuracy of the analyzed data, one must consider the "cleanliness" of the signals obtained.

As Dr. Schulman stated, "a continuous-wave Doppler system provides an image of all vessels which are crossed." In this context it is practically impossible to obtain a clean signal through a probe with a diameter of 12 mm (not gated, not ranged) directed either at the vaginal fornices or the right and left lower quadrants of the anterior uterine wall. This is simply due to the anatomic complexity of the vessels involved. From early gestation on, it has been shown in humans that the arterial tree around the fornices is very prominent and branching.[3, 4] If one remembers that there is an extremely rich network of venous plexuses accompanying them, the idea emerges that a beam attempting to measure movement of red cells in a single vessel is almost impossible. The solution seems to have been the arbitrary definition of "a pattern" ascribed to some individual vessels. There is continuous flow in the veins and pulsatile flow in the arteries; how can they be separated when the maximum caliber of the uterine arteries has been measured to be 3.6 ± 0.9 mm[5] and the beam is 12 mm wide? And what is the more deeply situated vessels' contribution to the spectrum? How much does the venous flow raise the baseline of your spectrum, and how is this set? The vaginal route does not seem to be the solution to the problem Dr. Schulman encountered in 1984 (see Reference 15 of article) when he stated that "uterine artery measurement is much more complex . . . it is difficult to be sure which you are measuring (referring to uterine, hypogastric, or arcuate-radial waveforms). As the uterus grows larger, this becomes an even more difficult technical problem."

The abdominal transducer does not have a simpler vascular tree to traverse in human pregnancy. Dr. Elizabeth Ramsey made the most elegant studies and reconstructions to date of the intertwining of the arteriovenous tree throughout the primate gestation (rhesus monkeys and humans).[6, 7] Clearly one cannot separate the movements of red cells in the various vessels with use of the technique here discussed. The nonplacental areas of the uterus are only moderately less complex, as shown by arteriograms.[8] This is a blind method relying only on an arbitrary pattern that, taken by faith, would represent the flow through an artery. Again here one must restate the questions: How are the authors sure of what signals they are recording? What are the guidelines to set the cursor for reading the systolic and diastolic values? How are artifacts recognized? What is the effect on blood flow of localized uterine contractions occurring throughout gestation? In these areas a 12 mm beam encompasses more arterial branches than in the vaginal fornix, and they are of lesser caliber. Unless these basic questions are satisfactorily answered, there is no point in discussing the results offered to us.

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DR. FRANK C. GREISS, JR., Winston-Salem, North Carolina. Basically I have all of the concerns that Dr. Cibils has. I have just one other comment. The sensitivity and specificity that Dr. Schulman showed indicate that whatever he is measuring is an apparent guide in terms of the development of pregnancy-induced hypertension.

DR. CHARLES BRINKMAN, Los Angeles, California. I had an opportunity when I was in Britain to see some of this work, and I certainly can agree with Dr. Schulman that the pattern recognition is possible. Even with an imaging technique and coupled pulse Doppler, one can pick out the various vessels just by the patterns.

The concern I have is the location from which you taken these various recordings. As we all know, the placenta of a hypertensive patient has varying degrees of infarction. If you look at it histologically, some of the vessels are markedly involved while others are much less involved. I wonder what the reproducibility of this technique is when you do not know from which vessel you are recording.

My question is: What is the reproducibility of this method in the same patient? I have great concern that each measurement may be from a different vessel, so I think reproducibility is an important question with this particular technique.

This is a very exciting study, and I think that as the technology catches up, it may become clinically useful.

DR. SCHULMAN (closing). It is not unique in science to raise theoretical objections to experiments, but the proof of the study is the experimental data and its reproducibility, so despite the objections, we, having done more than a thousand of these determinations, have little doubt about what we are seeing and about what we are measuring.

Two other laboratories, Dr. Campbell's and that of Dr. Trudinger in Australia, have obtained similar measurements; although their studies were carried out primarily in the last 5 weeks of pregnancy, the data are essentially identical.

Obviously, we took it one step further in describing an evolutionary process and the clinical outcomes.

In terms of the basic experiments, we certainly look forward to those being done. One chooses an experimental approach. One also chooses the subject to work on, animal versus human. The foundation for this work would not be answered by use of a flowmeter, since velocity is derived by dividing flow by the area distal to the point of measurement, for example, the resistance. Velocity is an understandable concept and will stand on its own as an important physiologic observation.

It has been our belief that the human data which are spilling out from half a dozen other laboratories around the world and particularly in the umbilical velocity work, are so consistent and so reproducible that I do not think it is necessary for us to undertake animal studies, but we certainly look forward to other people doing it. We think there is important human information that can be obtained immediately.

How does one obtain a clear signal? There are two ways to differentiate a vein from an artery. One can select a bidirectional system with a split screen, and it clearly shows flow going in two different directions. Second, the venous signal looks nothing like an arterial signal. It is a smooth, oscillating waveform. It does not have pulsations.

We record all our examinations on tape, so it is easy to review over and over again. It is a simple technical matter to move the probe to slightly different angles to obtain the maximum signal and the signal that you desire. It is easy to eliminate the venous signals in most circumstances. As one becomes experienced, one can recognize the overlying venous signal, and if it is interfering with the diastolic trough, one is obviously not going to use that particular signal in terms of describing the data.

I do not think this is a matter of faith. I have seen enough apples and oranges to be reasonably convinced that I know what they are when I see them.

We have with the probe directly on the vessel done this intra-abdominally in enough circumstances to be convinced that we are reasonably accurate about what we are seeing.

Regarding Dr. Greiss' comment, I did not mean to imply that I had determined the etiology of preeclampsia. I have simply demonstrated another associated physiologic event that occurs months to weeks before the clinical picture presents itself.

In answer to Dr. Brinkman's comments, it is possible to do many things with these probes. For example, in the early second trimester of pregnancy, you will see differences in the arcuate vessels and get different ratios. If you have the woman tilted slightly, you can move the probe slightly laterally and in many circumstances can follow the uterine artery right out to the iliac vessel.

The proximal uterine represents the summary of what is going on in all the arcuates that are involved, but once you get past a certain gestational age, a period we have identified as being about 26 weeks, they all measure out the same.

The intraobserver error is about 6%. When it is done day after day after day by the various people who work with us, we obtain the same results. Two nurses do most of our measurements at the present time.

Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study

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Factors related to the risk of developing clear cell adenocarcinoma in diethylstilbestrol-exposed women were investigated in 156 patients in the United States with clear cell adenocarcinoma and documented diethylstilbestrol exposure (Registry cases) and 1848 diethylstilbestrol-exposed women of similar age without cancer (National Cooperative Diethylstilbestrol Adenosis Project). Diethylstilbestrol dosage patterns, the use of other hormones, mother's age and pregnancy history, and daughter's birth month, birth weight, and age at menarche were compared. The relative risk was higher for those whose mothers began diethylstilbestrol before the twelfth week of pregnancy and for those who were born in the fall (winter conception). Maternal history of at least one prior spontaneous abortion elevated the risk. Supportive evidence for the difference in birth month distribution was also found by a comparison with United States vital statistics. Each factor was confirmed to be significant in a multivariate logistic model that examined all of them with region and year of birth adjusted. (Am J Obstet Gynecol 1986;154:814-22.)

Key words: Intrauterine diethylstilbestrol exposure, risk factors for clear cell adenocarcinoma

The association between intrauterine exposure to diethylstilbestrol and the subsequent development of clear cell adenocarcinoma of the vagina and cervix was established in 1971.[1-3] Subsequently a registry was founded for the study of these tumors in women born after 1940, irrespective of the maternal medication history. Information concerning the epidemiologic, clinical, and pathologic aspects has been covered in a number of reports[4-7] and currently more than 500 cases are accessioned. Diethylstilbestrol-associated cases make up approximately two thirds of the accessioned cases, most of which originated in the United States; additional cases have been reported from Canada, Mexico, Western Europe, Australia, and Africa. Previous findings indicated that the youngest diethylstilbestrol-exposed patient to develop clear cell adenocarcinoma was 7 years of age at the time of diagnosis, and a marked rise in the age incidence curve occurred at age 14 years. Evaluation of the incidence of diethylstilbestrol-associated cases in women born in the peak years of diethylstilbestrol use indicated that the increase in the occurrence of clear cell adenocarcinomas paralleled the changes in annual sales of 25 mg tablets by one large diethylstilbestrol drug manufacturer.[4]

In a preliminary study of diethylstilbestrol-exposed clear cell adenocarcinoma cases and control subjects the pattern of maternal diethylstilbestrol ingestion suggested that the risk of development of clear cell adenocarcinoma was higher among those whose mothers began diethylstilbestrol earlier in pregnancy.[6] This report was based on univariate frequency distributions and it was not possible at that time to control for other maternal, host, or exposure variables or to test for interactions among them.

The current study was undertaken to try to delineate the specific features of diethylstilbestrol exposure that may predispose to the occurrence of clear cell adenocarcinoma and to ascertain if there are identifiable maternal or host characteristics that affect the risk of appearance of this cancer. To evaluate these questions a case-control study has been undertaken in which data on diethylstilbestrol-exposed individuals with and without clear cell adenocarcinoma were available for analysis. The data allow multivariate analysis of potential risk factors and their interactions.

Methods

Initial classification of cases. For cases of clear cell adenocarcinoma, duplicate copies of hospital records and physician notes were obtained, including the obstetric records when available. Slides of the tumor and

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operative specimens were reviewed by Dr. Robert E. Scully or colleagues* in the Department of Pathology, Massachusetts General Hospital, Boston.

For diethylstilbestrol-associated cases details of the maternal history are recorded, including the level of precision regarding the time in pregnancy when diethylstilbestrol was begun and the duration and dose of therapy. For example, if the date of the last menstrual period and the exact date of starting diethylstilbestrol were known, the information regarding the time of initiation of diethylstilbestrol during pregnancy (in days from the last menstrual period) would be recorded as accurate to 1 day. Alternatively, if it was known that the drug was started in a certain week of the pregnancy and the exact date of the last menstrual period was available, then the information would be classified as being accurate to within 1 week. For time of starting diethylstilbestrol during pregnancy the data were classified as being accurate to within 1 day, 1 week, 2 weeks, 1 month, 2 months, or one trimester. Similar refinements were used for duration of therapy, and dosages were recorded as accurate to 1.0, 10, 100, 1000, or >1000 mg.

In all of the cases in the current study, written information from the prenatal, hospital, or pharmacy records was available. In the analysis of diethylstilbestrol exposure patterns the level of precision for day diethylstilbestrol was begun was ≤2 weeks, for duration of diethylstilbestrol use the precision also was ≤2 weeks, and for total dose given the precision was ≤100 mg.

The control subjects consisted of diethylstilbestrol-exposed women without cancer who were identified through physicians' records by the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project.[8, 9] The national DESAD project began in 1974 to identify women exposed in utero to diethylstilbestrol and has provided annual examinations and questionnaires in order to investigate the long-term effects of diethylstilbestrol exposure. Five centers have participated in the project (Baylor College of Medicine, Houston; Cedars-Sinai Medical Center, Los Angeles; Gundersen Clinic, LaCrosse, Wisconsin; Massachusetts General Hospital, Boston; Mayo Clinic, Rochester, Minnesota).† Prenatal records were reviewed to identify individuals who were then traced and recruited for follow-up (record review participants). Entries in the prenatal record regarding diethylstilbestrol medication (last menstrual period, date of initiation, dose, dates of change in dose, and dates of ending medication) were recorded. Other DESAD project participants were referred by physicians or were self-referred, but only those identified by record review were included in the present case-control comparison.

Table I. Variables compared in cases and controls

Daughters
Birth year, birth month
Region, state of birth
Race
Birth weight
Age at menarche
Diethylstilbestrol exposure in utero
Day diethylstilbestrol began
Duration of diethylstilbestrol exposure
Total dosage of exposure
Progesterone in addition to diethylstilbestrol
Mothers
Age
Prior No. of pregnancies
Prior spontaneous abortions
Prior stillbirths
Prior infant deaths

The record review daughters from the DESAD project provide the least biased data in terms of the accuracy of the information on exposure to diethylstilbestrol and are the most representative data available on diethylstilbestrol-exposed mothers and daughters in the United States.

The criteria for recording diethylstilbestrol dosage and other study variables were reviewed at the beginning of this investigation by DESAD and Registry staff to determine which variables were recorded in a similar fashion by the two groups and thus could be accurately transformed into identical variables for precise comparisons (Table I).

Maternal reproductive history for both cases and controls was ascertained from the prenatal records and from questionnaires filled out by the mothers. Numbers of prior pregnancies, prior spontaneous abortions (loss before the twentieth week), and prior perinatal loss (stillbirths and infant deaths) were recorded. Also recorded were the mother's age, daughter's birth weight, and medications other than diethylstilbestrol prescribed in the pregnancy. Although data on complications in the index pregnancy such as prior infertility, bleeding, cramping, diabetes, or hypertension were included in both groups, the information was recorded with the use of different criteria and an accurate comparison of these variables could not be performed in the current investigation.

The daughter's age at menarche, marital status, oral contraceptive use, and pregnancies were recorded at the time of follow-up examinations and by question-

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Table II. Distribution of selected demographic characteristics for diethylstilbestrol-exposed daughters with and without clear cell adenocarcinoma

Characteristic	Cancer cases		Controls	
	No.	%	No.	%
Birth year				
1945-1949	11	7.1	347	18.8
1950-1954	69	44.2	704	38.1
1955-1959	63	40.4	481	26.0
1960-1965	13	8.3	316	17.1
Total	156	100.0	1848	100.0
Region of birth*				
South	35	22.6	357	19.3
Northeast	48	31.0	342	18.5
North Central	37	23.9	592	32.0
West and Rocky	35	22.6	557	30.1
Total	155	100.0	1848	100.0
Race				
White	149	96.1	1830	99.0
Black	6	3.9	12	0.7
Other	–	–	6	0.3
Total	155	100.0	1848	100.0

*Grouping of states as divided into regions defined by the United States Bureau of the Census.

naires. The information on oral contraceptive use was recorded somewhat differently in the two studies, and the relatively small number of registry cases with comparable lifetime experience of oral contraceptive use and pregnancies before the diagnosis of cancer did not allow meaningful comparison.

Estimates of risk factors. Both the cases and controls in this study were limited to daughters born in the United States with written documentation of diethylstilbestrol exposure. The birth years of the cases and controls differed significantly (Table II). Because of the changes in diethylstilbestrol prescribing patterns during the years between 1941 and 1971, only those control subjects who were born during the same period as the cases (1945 to 1965) were included in the analyses, and all the multivariate analyses were adjusted for birth year.

The distribution by region of birth of the cases and controls also differed significantly, mainly because of the fact that the control subjects came from only five examining centers while the cancer cases have been reported from all areas throughout the United States. In order to take this into account, all multivariate analyses were adjusted for region of birth, as defined by the United States Bureau of the Census. Separate analyses within each region were done and the results were similar to those reported in this paper for all of the variables.

Initial univariate comparisons of the frequency distributions for the cancer cases and DESAD controls were done with the use of χ^2 tests (two-sided 0.05 level). Continuous variables were also compared with independent t tests. Multiple logistic discriminant analyses, adjusted for region and year of birth, were then used to identify significant variables.[10] All of the logistic analyses were done at the 0.05 level of significance. Stepwise procedures were used to identify the most significant variable from the set being analyzed. The analyses were done in two stages, looking first at diethylstilbestrol exposure variables. Then the maternal and host variables were examined with correction for the effects of the diethylstilbestrol exposure variable that was found to be significant, for instance, "day DES began" (see Results). This procedure allowed separate evaluation of the additional effects of maternal and host variables on diethylstilbestrol-associated cancer risk.

Stage 1: Evaluation of diethylstilbestrol effects. The effect of the day the mother began diethylstilbestrol and the duration and the total dose of diethylstilbestrol exposure were analyzed, with the region and year of birth controlled. The exposure variable that we found to be significant was the day diethylstilbestrol was begun in pregnancy. This variable was later categorized into four groups (before seventh week, seventh to eighth week, ninth to twelfth week, and ≥13 weeks) in order to present more clearly the risks associated with starting diethylstilbestrol in each interval during pregnancy (Table III). The effects of the duration and total dose of diethylstilbestrol were not significant, perhaps because of the small number of cases with precise and complete information on these variables. Thus the duration of diethylstilbestrol therapy during pregnancy and the total dosage ingested by the mother were both deleted from the analyses. The adjusted relative risks (odds ratio) presented in Tables III, IV and V were calculated from the logistic model and refer to the risk in one category versus a risk of 1.00 assigned to the last category. For example, in Table III the risk associated with the mother's beginning diethylstilbestrol before the seventh week was compared to the risk for those whose mothers began diethylstilbestrol at 13 weeks or later.

Stage 2: Evaluation of other maternal and host characteristics. In the second stage, stepwise logistic discriminant analyses were then done for other maternal and host characteristics in order to determine which variables significantly increased or decreased the risk, with the effect of day of beginning diethylstilbestrol exposure taken into account. Three control variables—region of birth, year of birth, and day diethylstilbestrol began—were all retained in the model for the analysis of the other characteristics. Initially any variable that was continuous was so treated, then later categorized into two to four groups in order to display the results

Table III. Relative risk for time of starting diethylstilbestrol in pregnancy

Diethylstilbestrol began	*Cases*	*Controls*	*Unadjusted relative risk*	*Adjusted relative risk**	*95% Confidence interval*
Before week 7	21	188	4.52	3.65	1.84-7.25
Week 7-8	35	260	5.45	4.84	2.63-8.90
Week 9-12	41	449	3.76	3.48	1.94-6.25
Week 13 or later	17	688	1.00	1.00	
Total	114	1585			

The risk in each time interval is compared to a risk of 1.00 for the last time interval.
*Adjusted for region and year of birth.

in Table IV in a more simplified manner. The adjusted relative risks shown are calculated from the logistic model.

Finally, a stepwise logistic discriminant analysis was done, with incorporation of the significant variables from the maternal and host discriminant analyses, to estimate the effect of each on the relative risk of developing clear cell adenocarcinoma (Table V). The cases included in this final model had accurate information for all of these variables.

Supplemental analyses with cross-tabulation by the Mantel-Haenszel method yielded estimates of odds ratios consistent with those reported in Tables III, IV, and V.

For the comparison of the birth months of the case subjects to birth months expected in the general population, United States Census data were examined for each year, for the race and state of birth of each case subject. For example, for a cancer patient who was white, born in 1951, in California, the 12 probabilities of a white individual being born in January, February, March, etc., in California, in 1951, were calculated from the census figures. The expected probabilities of birth in January, February, March, etc., were added up for all the cases to calculate the mean expected probabilities shown in Table VI and Fig. 1.

Results

Diethylstilbestrol exposure patterns. Written documentation of diethylstilbestrol exposure was available on 156 patients born in the United States with clear cell adenocarcinoma and 1848 control subjects born in the United States without cancer (born 1945 to 1965), from the "Record Review" category of the DESAD project. Table II displays the demographic characteristics of each group. The significant differences between these groups with respect to year ($p = 0.0001$) and region of birth ($p = 0.0004$) were taken into account by systematically adjusting for these two variables in the results presented.

Evaluation of the maternal records for the cancer cases indicated that the most precise and complete information regarding diethylstilbestrol exposure was the day medication was started during pregnancy. The duration of treatment and the total dosage of diethylstilbestrol ingested were evaluated and their effects were not significant, perhaps because of the small number of cases with precise and complete information. Stepwise logistic discriminant analysis found the day diethylstilbestrol was started to be significant ($p < 0.05$). The results presented are limited to those cases for which the timing of diethylstilbestrol treatment during pregnancy could be ascertained within 2 weeks of the last menstrual period. The comparative distribution of these cases and controls is shown in Table III. The relative risk, adjusted for differences in birth year and region, is also shown with 95% confidence intervals.

In all of the cancer cases except one, the mother began diethylstilbestrol before the nineteenth week of pregnancy. In that case the record indicates diethylstilbestrol was begun in the thirty-second week of pregnancy (150 mg given for an unknown length of time). The relative risk of cancer development is lowest for those daughters whose mothers began diethylstilbestrol after the twelfth week of pregnancy. The apparently smaller relative risk during weeks 0 to 7 was investigated further by examining the histories of the 21 cases in which diethylstilbestrol was begun before the seventh week of pregnancy. Only two of the mothers began diethylstilbestrol in small doses before conception for infertility therapy and continued taking it, while the other 19 began diethylstilbestrol as pregnancy therapy during the fifth or sixth week. Supplemental analysis with cross tabulation by the Mantel-Haenszel method indicated some regional variation in the risk level for each of the time intervals; however, it confirms that the risk is lower for those whose mothers began diethylstilbestrol late in pregnancy.

Additional risk factors—Maternal and host characteristics. In addition to investigating the role of diethylstilbestrol exposure, a second goal of this study was to determine if any maternal or host characteristics either significantly decreased or increased the risk of developing clear cell adenocarcinoma. Thus all analyses

Table IV. Relative risk for maternal and host characteristics

Characteristic	*Cases*	*Controls*	*Unadjusted relative risk*	*Adjusted relative risk**	*95% Confidence interval*
Mother's age at time of index pregnancy					
<26 yr	35	597	0.97	0.97	0.54-1.75
26-30 yr	37	635	0.97	1.05	0.61-1.81
>30 yr	36	598	1.00		
Total	108	1830			
Prior pregnancy					
≥1	119	1189	1.79	1.59	0.89-2.85
0	21	376	1.00		
Total	140	1565			
Prior spontaneous abortion for those who had prior pregnancy					
≥1	91	626	2.80	2.46	1.45-4.20
0	27	520	1.00		
Total	118	1146			
Prior stillbirth or infant death					
≥ 1	13	113	0.93	1.05	0.53-2.09
0	105	846	1.00		
Total	118	959			
Progesterone given with diethylstilbestrol					
Yes	33	437	0.88	1.27	0.79-2.06
No	121	1411	1.00		
Total	154	1848			
Steroidal estrogen given with diethylstilbestrol					
Yes	2	53	0.45	0.66	0.15-2.83
No	152	1795	1.00		
Total	154	1848			
Season of birth					
Fall (Sept.-Nov.)	49	416	1.93	2.21	1.25-3.91
Winter (Dec.-Feb.)	39	416	1.53	1.65	0.91-2.99
Spring (Mar.-May)	38	524	1.15	1.14	0.62-2.10
Summer (June-Aug.)	29	474	1.00		
Total	155	1848			
Birth weight					
≤5.5 pounds	20	191	2.70	2.37	1.23-4.57
>5.5 pounds	64	1648	1.00		
Total	84	1839			
Age at menarche					
<13 yr	69	779	1.23	1.27	0.85-1.88
≥13 yr	76	1059	1.00		
Total	145	1838			

*Adjusted for region and year of birth and early or late exposure to diethylstilbestrol (day began ≤63 or >63).

investigating maternal and host characteristics are adjusted not only for region and year of birth but also for time diethylstilbestrol was begun.

Table IV presents the relative risk estimates for the maternal and host characteristics. Most of the comparisons did not yield significant differences. Progesterone and steroidal estrogen were given in addition to diethylstilbestrol in comparable proportions of cases and controls. Distributions of mother's age and age of menarche were the same in both groups. There are statistically significant adjusted relative risks for the fall season of birth (reflecting winter season of conception) and for maternal history of at least one spontaneous abortion.

When the data were evaluated for daughters with premature birth (infant's birth weight <2500 gm), it was found that this group had an increased risk of developing clear cell adenocarcinoma, with region and year of birth and time of exposure to diethylstilbestrol adjusted (relative risk = 2.37); however, birth weight information was available for only 84 of the cancer cases and only one third of the cases (54) had information on the four variables (time of diethylstilbestrol exposure, season of birth, history of spontaneous abortion, and premature birth). The small numbers of cases with complete information on all of the variables prevented meaningful inclusion of premature birth as a factor in the subsequent multivariate logistic model.

To determine the relative importance of the factors identified in Table IV on the risk of developing clear

Table V. Relative risks* for most important risk factors

Factor	*Relative risk*	*95% Confidence interval*
Exposure to diethylstilbestrol		
Early (≤63 days)	1.97	1.27-3.06
Late (>63 days)	1.00	
Season of birth		
Fall	1.90	1.22-2.95
Other	1.00	
Prior spontaneous abortions for those who had prior pregnancy		
Yes	2.36	1.38-4.04
No	1.00	

*Adjusted for birth year and region. All factors had to be known for the case to be included in the logistic model.

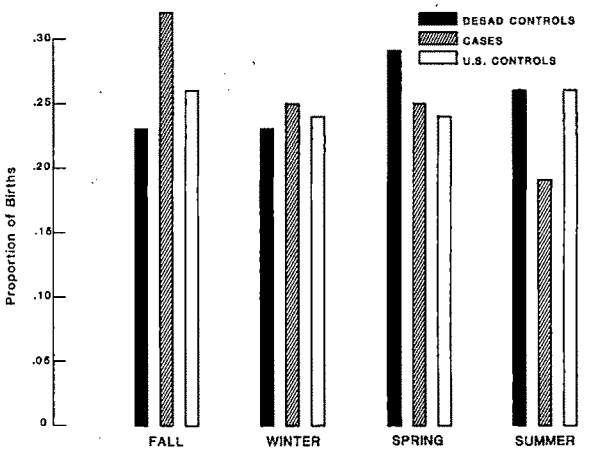


Fig. 1. Season of birth for controls, cases, and United States population for the states, years, and race of cases.

cell adenocarcinoma, a final discriminant analysis was performed, which included the day diethylstilbestrol was begun (day 63 or earlier or later than day 63), season of birth (fall or other), and prior spontaneous abortion. The adjusted relative risks and their confidence intervals are presented in Table V. There was no statistically significant interaction among the three variables considered (time of beginning diethylstilbestrol, season of birth, and prior spontaneous abortion).

The distribution of birth months for the cases was also examined in comparison to the expected distribution calculated for each case with the use of United States National Office of Vital Statistics data for the year of birth, state, sex, and race of each case to ascertain if the differences noted between the cases and controls also existed when the distribution of cancer cases was compared to that in the general United States population. The difference from the expected distribution is shown in Table VI ($\chi^2 = 19.53$, 11 df, $p = 0.052$). This result is supportive of a difference in birth month distribution in the cancer cases in the months of September through December, corresponding to conception in the "flu" months of December through March. Fig. 1 displays the distribution of season of birth for cases and controls and for United States women born in the same states and years as the cases. The controls are seen to be representative of the United States natality experience as reported by the National Office of Vital Statistics.

Table VI. Distribution of birth month of cases of clear cell adenocarcinoma and expected distribution from United States National Office of Vital Statistics

Month	*n*	*Probability for cases**	*Probability for United States vital statistics*†*
Jan.	7	0.0458	0.0812
Feb.	15	0.0980	0.0764
March	12	0.0784	0.0830
April	17	0.1111	0.0772
May	8	0.0523	0.0802
June	5	0.0327	0.0810
July	16	0.1046	0.0883
Aug.	8	0.0523	0.0904
Sept.	16	0.1046	0.0890
Oct.	13	0.0850	0.0875
Nov.	19	0.1242	0.0816
Dec.	17	0.1111	0.0847
Total	153	1.0000	1.0000

*$\chi^2 = 19.53$, 11 df, $p = 0.052$.

†United States vital statistics for births in each individual's state, race, and year of birth. Data for 19 cases were available only for women of all races for that particular state and year. Data for two cases were unavailable.

Comment

These results of a comparison between diethylstilbestrol-exposed women with and without clear cell adenocarcinoma indicate several of the factors that are associated with the risk of developing this cancer. The Registry for Research on Hormonal Transplacental Carcinogenesis has collected data on most of the cases of clear cell adenocarcinoma of the vagina and cervix that have occurred in the United States in women born since 1940. This collection of cases is the sole source of centralized data available for the investigation of these tumors. The DESAD project, from which the controls were drawn, has participating centers in four areas of the country, mainly associated with large teaching hospitals. The DESAD centers differed from each other on the variables of DES exposure, birth weight, mother's age, and mother's number of prior pregnancies and spontaneous abortions so that regional variation must be taken into account. The centers may not represent the entire country, but the pooled data give a good approximation and provide the most representative comparison data available. Analyses done within each

region of the country are consistent with the results presented, and the inferences did not differ with or without adjustment of the region of birth.

The results of the analyses on diethylstilbestrol exposure patterns, adjusted for differences between the clear cell adenocarcinoma cases and DESAD controls in region and year of birth, indicate a lower risk for cancer among those women whose mothers began diethylstilbestrol after the twelfth week of pregnancy. This association had been indicated in a prior comparison of the distribution of the week diethylstilbestrol was begun between diethylstilbestrol-exposed clear cell adenocarcinoma cases and controls.[6] In many of the prenatal records reviewed there was sparse and imprecise information available on the duration and the total dosage of DES expsoure in pregnancy and the limited nature of the data prevented conclusions being drawn about the role of these exposure variables in the risk of clear cell adenocarcinoma. Diethylstilbestrol prescribing patterns varied in different parts of the country and in different years and the exposures differed among the DESAD centers and between the regions described in these results.[8] There was regional variation in the amount of risk elevation associated with early time intervals of beginning diethylstilbestrol but the regional analyses confirm the lower risk for those whose mothers began diethylstilbestrol later than the twelfth week of pregnancy, as presented in the pooled national data (Table III). The risk does not appear to decline progressively concomitant with diethylstilbestrol exposure beginning later in pregnancy; that is, the risk is greater for those whose mothers began diethylstilbestrol in weeks 7 and 8 than for those whose mothers began diethylstilbestrol before week 7. It is not possible from the available data to explain this seemingly paradoxical result. For example it is conceivable that diethylstilbestrol dosage may also be a significant risk factor that was not detected in the current analyses because of the lack of sufficient cases with precise data. If this were true and a high proportion of the mothers who started diethylstilbestrol before week 7 also received smaller dosages of diethylstilbestrol then the observed result of a smaller risk before the seventh week would be obtained. Numerous other speculative explanations exist.

It is not possible to determine from these data if the elevated risk for clear cell adenocarcinoma development among those whose mothers began diethylstilbestrol before the twelfth week in pregnancy is directly related to early diethylstilbestrol exposure or mediated by the development of vaginal adenosis and associated epithelial changes that occur more frequently in women exposed early to diethylstilbestrol and also to higher doses.[9] Areas of vaginal adenosis with the most extreme atypia and a tuboendometrial glandular pattern are frequently topographically associated with tumor areas.[11]

Among the other maternal and host characteristics investigated, the mother's history of prior spontaneous abortion, daughter's low birth weight, and fall season of birth were found to be significant risk factors in addition to early diethylstilbestrol exposure.

The elevated rate of prior spontaneous abortion among the mothers whose daughters developed clear cell adenocarcinoma raises the possibility that an inherited genetic predisposing factor may have played a role in some of these cases. In the original case-control studies in 1971, comparing women with clear cell adenocarcinoma and controls, higher rates of complications such as bleeding and prior spontaneous abortion had been noted in the maternal histories of the cancer cases, as was a higher incidence of ingestion of diethylstilbestrol, which was frequently prescribed for these pregnancy complications.[1,2] It has been suggested that the maternal history of spontaneous abortion itself could result in an individual that is more susceptible to clear cell adenocarcinoma and this susceptibility may have been a more important factor in cancer development that the association with exposure to diethylstilbestrol.[12] The current case-control results do not support this explanation. Although the maternal spontaneous abortion history is a risk factor, the time of exposure to diethylstilbestrol during the pregnancy remains significant even after the maternal history of prior spontaneous abortion is adjusted (Table V).

The higher frequency of birth weight <5.5 pounds in the cancer cases compared to the DESAD controls may also suggest maternal complications as predisposing factors in cancer development or an inherent disadvantage in premature babies in resisting subsequent carcinogenic factors such as viral infection. Such interpretations must be tempered however, since data on birth weight were available in only 84 of the 156 cases studied.

The analyses of month of birth show a prevalence of fall births in the cancer cases whether compared with births of the DESAD controls or to the birth month distribution expected from United States census figures. Patients born in the fall will have been conceived primarily in the winter months during the prevalence of influenza virus. The regional distribution of cases and controls between cooler winter areas (north central and northeast) and warmer areas (south and west coasts) was similar. Viruses have been associated with some cancers and animal studies have suggested immunologic deficiency after diethylstilbestrol exposure in rodents.[13] Thus a viral role in clear cell adenocarcinoma is suggested but these data do not permit more than speculation in regard to a potential role. It is also conceivable that the observed differences are artifactual

and not indicative of any influence on the development of clear cell adenocarcinoma.

It appears that the time of exposure to diethylstilbestrol during pregnancy is a critical factor in the risk for clear cell adenocarcinoma and that, after its effect is adjusted, premature birth, fall birth season, and a maternal history of spontaneous abortions also appear to be implicated.

We are grateful to all of the investigators of the DESAD project for their help in making these data available, and to Diane Anderson of the Registry at the University of Chicago for valuable assistance.

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Discussion

DR. HUGH M. SHINGLETON, Birmingham, Alabama. I congratulate Dr. Herbst and his associates for their continuing effort to identify the specific risk factors for clear cell adenocarcinoma in diethylstilbestrol-exposed women.

Some of my remarks were provided by Seng-Jaw Soong, Ph.D., biostatistician for the Comprehensive Cancer Center at the University of Alabama at Birmingham. We compliment the authors for the excellent statistical analysis using univariate and multivariate analytical procedures, in particular the logistic regression model.

In considering the manuscript, one immediately runs into problems of numbers. Missing data have undoubtedly limited flexibility and usefulness of the multivariate analysis and the interpretation of results. Starting with 500 cases of adenocarcinoma accessioned into the registry, one must first delete the one third whose cancers are not diethylstilbestrol associated. In further limiting the analysis to those with the most data on 15 variables (ten daughter and five maternal related) one comes down finally to 156 individuals or less. Indeed, the totals considered in the various tables range from 84 to 156, nine different totals being portrayed. The most important factor, time of exposure to diethylstilbestrol, was determined on analysis of data from only 114 cases. Record-review daughter controls from the DESAD project were selected because of the availability of the "least biased and most representative" data available on diethylstilbestrol-exposed mothers and daughters in the United States. However, since only a subset of these data are used in this multivariate analysis, are the subsets unbiased and representative?

In regard to Table V, we were unable to determine the number of cases and controls that were used for this final analysis. Concerning the relative risks shown for early and late exposure to diethylstilbestrol, we felt that the groupings above and below 63 days were somewhat arbitrary and difficult to reconcile with the groupings in Table III, which divided the exposure in the categories before the seventh week of pregnancy, the seventh to the eighth week, the ninth to the twelfth week, and 13 weeks and beyond. In regard to the season of birth, where the fall season was compared with the remaining seasons, we also could not determine the reason for selecting the fall for comparison when on Table VI February, March, and April seemed equally attractive. Since fall deliveries imply a winter conception, was consideration given to the possible influence of other drugs taken during the winter season, especially over-the-counter drugs? Furthermore, was patient compliance in taking the diethylstilbestrol itself considered?

In summary, even with the limitations, this undoubtedly is the best information available, or likely to be available, on the subject. I would like to ask Dr. Herbst two questions: (1) How many cases and controls were included in the final analysis (Table V)? (2) Considering the very low relative risk of women who received steroidal estrogens along with the diethylstilbestrol, might the estrogens actually be a protective factor?

Were the subsets unbiased after we got through looking at those we had the information on? Obviously,

there is no way we can answer that. We have to assume that there is no reason for this type of precision to introduce bias into the analysis, but that is speculation.

Could drugs other than those we talked about with the viruses and the season of birth be involved in the genesis of this entity? The answer is, of course, they may be, but we do not have any information so we can just guess.

Dr. Shingleton wondered why 63 days was chosen in the final model. The reason for that is half the patients' mothers started diethylstilbestrol before the sixty-third day and half after the sixty-third day. Any other cutoff point could have been picked. The results would have been exactly the same, but that is why that point was chosen.

For our definition, the fall season of birth means September, October, and November, so we did not choose the 3 months that Dr. Shingleton mentioned. Again that is arbitrary, but that is the way we did it.

I do not believe steroidal estrogens have a protective effect. They were used proportionally about the same in both the cancer cases and in the noncancer cases. Although the relative risk was less than 1, the 95% confidence limits overlapped 1, and I do not believe that that is a biologically important fact.

As far as Table V was concerned, there were 104 cases in that particular analysis and 1323 controls.

As far as the other questions, Dr. Jones asked us about family history. We have looked into that in some of our other studies. We did not look into it in this study because those data were not recorded, and all we can say so far, particularly in the first study that we did in 1971, is that it was not an important factor.

Dr. Howard Jones III, Nashville, Tennessee. In this paper the authors have evaluated the relationship between cancer and the effects of diethylstilbestrol in both the mothers and the daughters and associated the fertility problems but have not considered in their analysis other factors that we know to be related to cancer, such as a family history of cancer, smoking, and so forth. I wonder if they had considered including these factors in their analysis of cancer risk in these patients?

Dr. Charles E. Flowers, Birmingham, Alabama. Many of these patients were from Boston, where Dr. Priscilla White was administering hormone supplements to her diabetic patients. Could this have been of any significance?

Dr. E. Stewart Taylor, Denver, Colorado. I was interested in the analysis that had to do with the increased number of spontaneous abortions that occurred in patients whose offspring had an increased incidence of adenocarcinoma of the vagina.

I wonder if that is not because patients with a previous history of many abortions came early and had enthusiastic treatment with large doses of diethylstilbestrol compared with those who just casually received the drug for other reasons.

Dr. Richard K. Miller, Rochester, New York. With diethylstilbestrol exposure, the medical community has been primarily concentrating on prenatal factors that may modify the incidence of vaginal tumors. Is there any possibility that postnatal factors and complications may also be important? In particular, have you, Dr. Herbst, investigated the relationship between prenatal diethylstilbestrol exposure and postnatal use of oral contraceptives?

Dr. Herbst (Closing). First, regarding the issue of the number of cases, Dr. Shingleton is absolutely correct. We were limited by the numbers that were available. In other words, we did not do the analysis unless there was written documentation of the diethylstilbestrol exposure, so that resulted in the 156 cases. Then, in some of the cases, all of the information was just not available.

We did require a high precision for the data to be analyzed. For example, for the analysis on the time in pregnancy the diethylstilbestrol was started, we either knew the precise day it started or we knew that information to be within 2 weeks of the last menstrual period. If there was written verification that the mother took the diethylstilbestrol but we did not know within 2 weeks of the last menstrual period when she started it, she could be in some of the analyses but not the time started analysis.

I would say the same thing about oral contraceptives. I cannot say they are not important. We have no information that they are important. We have looked at that, and I think it may be worthwhile to point out that among many of these cases, the young women were virginal at the time they developed cancer. Not only was there no oral contraceptive exposure, there had not been any sexual exposure, so although oral contraceptives might have some role, there is no suggestion that they do at all.

As far as Priscilla White's diabetic series is concerned, they have been examined in Boston. They do have vaginal adenosis and other diethylstilbestrol-associated changes. I am not aware of any cases of cancer in that group. However, if my memory is correct, there were about 700 daughters in that series and the risk of the cancer developing is of the order of ≤ 1 in 1000; therefore, it is not surprising to have a series of 700 individuals and have no cases of cancer. However, they definitely have the diethylstilbestrol-associated changes.

As far as spontaneous abortions are concerned and Dr. Taylor's statement about the mothers receiving diethylstilbestrol earlier in pregnancy, I think that is clearly a possibility. In this particular analysis, the history of spontaneous abortion was present in both the controls and the cancer cases, and for some reason spontaneous abortion was more frequent in the cancer cases. For that reason it turned out to be statistically important, so maybe the old idea that was brought up many years ago, of the bad egg, may be a possibility, but again it is just speculation.

Effect of induced oligohydramnios on fetal lung development

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To investigate the impact of oligohydramnios on fetal growth, development, and lung function, amniotic fluid was shunted from alternate gestational sacs into the maternal peritoneal cavity at 23 days' gestation in the fetal rabbit. Uninstrumented fetuses served as controls. Oligohydramnios was confirmed at the time of planned cesarean delivery on either day 26 or 28. Fetuses subjected to prolonged oligohydramnios had significantly decreased body weight at 26 ($p = 0.002$) and 28 days ($p = 0.007$). Similarly, lung weight was decreased at 26 ($p = 0.02$) and 28 days ($p = 0.005$). There was a trend toward decreased maximum lung volume for instrumented fetuses at 28 days ($p = 0.07$). Deflation limbs of the pressure-volume curves revealed significantly increased air trapping for instrumented fetuses at 28 days. These studies suggest not only a detrimental effect of oligohydramnios on fetal body and lung weight but also a beneficial effect of premature rupture of membranes on fetal lung function. Analysis of phospholipid and histologic changes is currently under way. (Am J Obstet Gynecol 1986;154:823-30.)

Key words: Oligohydramnios, premature rupture of membranes, fetal growth, pulmonary hypoplasia

The definition of premature rupture of membranes is rupture of the amniotic sac that occurs before the onset of uterine contractions. This complication occurs in approximately 10% of pregnancies at term and in 0.7% to 2.0% of pregnancies before 37 weeks.[1] Although the management of premature rupture of membranes at term usually includes induction of labor, a conservative approach to the management of preterm premature rupture of membranes has been advocated in an attempt to decrease the incidence and severity of neonatal respiratory distress syndrome. Within 24 hours of premature rupture of membranes 80% to 90% of patients can be expected to be in labor.[2] However, when premature rupture of membranes occurs remote from term, a longer latency period can be expected. Mead[3] showed that in preterm pregnancies 20% to 25% of patients were undelivered after 7 days. Taylor and Garite[4] recently reported that in patients with premature rupture of membranes before fetal viability (16 to 25 weeks), 62.3% delivered within 1 week, 71.7% within 2 weeks, and 77.4% within 1 month.

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Chorioamnionitis, endomyometritis, and sepsis represent the major maternal risks associated with conservative management. Risk assessment for the fetus includes stillbirth, neonatal infection, respiratory distress, neonatal death, deformation syndrome, and pulmonary hypoplasia. If a conservative approach to the management of preterm premature rupture of membranes is taken and a prolonged latency period occurs, the suspected association between oligohydramnios and lung hypoplasia becomes increasingly important. Normal fetal lung development during pregnancy requires a critical volume of amniotic fluid.[5-6] Neonates delivered from pregnancies complicated by oligohydramnios often have severe pulmonary hypoplasia resulting in early death[3, 7, 8] or evidence of diminished growth.[9] Theoretically, the absence of amniotic fluid allows the uterus to exert continual pressure against the fetus, resulting in abnormal fetal growth and development. The loss of fetal intrapulmonary fluid acting as an internal stenting force provides an additional explanation for pulmonary hypoplasia resulting from preterm premature rupture of membranes.[10] The role of preterm premature rupture of membranes as an effective stimulus to accelerate functional pulmonary maturity in the fetus is controversial. Mead[3] found 14 studies in the literature demonstrating a decreased incidence of respiratory distress syndrome after prolonged premature rupture of membranes and 11 studies unable to show any such association.

To examine the implications of oligohydramnios resulting from premature rupture of membranes, we developed an animal model of chronic amniotic fluid leakage in the fetal rabbit. Our study was designed to in-

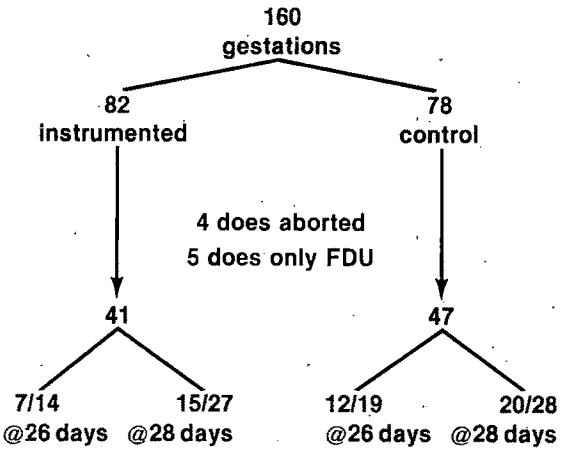


Fig. 1. Fetal loss rate associated with laparotomy and placement of Penrose shunt for chronic amniotic fluid leakage. *FDU*, fetal death in utero.

vestigate the association between oligohydramnios and alterations in fetal growth characteristics. In addition, the reported beneficial effect of premature rupture of membranes on fetal/neonatal lung function was assessed.

Material and methods

Twenty-three time-dated pregnant New Zealand white rabbits were purchased from a local animal breeder. On arrival at the Georgetown Animal Resources Facility on day 20 to 21 of pregnancy, each doe was weighed and housed in a separate cage. Guidelines established by the American Association for Accreditation of Laboratory Animal Care were followed during this study. Commercially available dry rabbit food and fresh water were provided ad libitum. On day 23 of gestation, after an overnight fast, the does were weighed and given an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). After the doe was sedated, the abdomen was shaved and the trachea was visualized and intubated with a 3.0 mm Portex endotracheal tube. Animals were restrained on the operating table and anesthesia was maintained with 1% to 2% halothane in oxygen. After the abdomen was prepared and draped, the uterus was exposed through a midline abdominal incision. The number of fetuses per uterine horn was noted. Hysterotomy and amniotomy were carried out on alternate gestational sacs. The uninstrumented littermates served as controls. Continued drainage of amniotic fluid into the maternal peritoneal cavity was assured by placement of a 2 cm segment of ¼ inch Penrose drain. The hysterotomy was closed with 1 or 2 simple stitches of 4-0 Prolene incorporating the Penrose drain to prevent displacement. The abdomen was closed in layers, and each doe was given 300,000 U of procaine penicillin subcutaneously. The doe was kept warm during recovery from anesthesia and returned to her cage where food and water were again provided.

The pregnancy was allowed to continue until either day 26 or 28, at which time the doe was weighed and sacrificed by intravenous sodium pentobarbital (60 mg/kg). The fetuses were delivered by rapid cesarean section and their tracheas clamped to prevent breathing. Death occurred shortly thereafter. The fetal neck was opened, and the trachea was dissected free. The trachea was opened and cannulated with a 19-guage tubing adaptor tied in place with 3-0 silk. The adaptor was connected to a system previously reported[11] that accurately records air pressure/volume curves. The experimental protocol consisted of inflating each set of lungs to 35 cm of water and maintaining this pressure for 4 minutes to obtain maximum lung volume. Three deflation-inflation cycles were performed at a pressure between 0 and 35 cm of water. Cycling times for each completed loop were between 2 and 4 minutes. In comparing these curves between different fetuses, we chose to analyze only the second inflation and deflation limb. The second loops appeared most reproducible in that they were essentially identical to the third loops. Air volumes were expressed as milliliters of air per kilogram of fetal body weight and milliliters of air per milligram of wet lung weight at maximum lung volume. Proportional volume changes during inflation and deflation were expressed as a percentage of maximum lung volume which was designated as 100%. Lungs that developed air leaks during the pressure/volume studies were eliminated from this phase of analysis.

On completion of the pressure/volume studies, the fetal body, lung, and liver were weighed to 0.001 mg. To standardize the organs to account for differences in body weight, ratios of lung and liver to body weight were calculated.

Two-factor analysis of variance was used to examine the proportional lung volume changes, and the unpaired Student's T test was used for all other statistical analysis; p values <0.05 were considered significant.

Results

Of the 23 does, one was not pregnant and one aborted before the surgical procedure. Following the intramuscular injection, one animal had a respiratory arrest and could not be resuscitated, leaving 20 animals for study. The mean number of fetuses per doe was 7.5 (range 4 to 11). In general, the fetuses were implanted in each uterine horn in equal numbers. Of the 160 fetuses noted at laparotomy on day 23, 82 underwent creation of the amniotic fluid fistula and 78 littermates served as controls. Four does aborted before scheduled cesarean delivery, and five were found to

Table I. Weight alterations resulting from induced oligohydramnios*

	Body weight		*Lung weight*		*Lung weight/ body weight*		*Liver weight*		*Liver weight/body weight*	
	n†	*gm*	*n*	*gm*	*n*	%	*n*	*gm*	*n*	%
26 days' gestation										
Control	12	19.87 ± 3.89	5	0.60 ± 0.14	5	2.92 ± 0.16	5	1.52 ± 0.53	5	7.18 ± 0.94
Amniotic fistula	7	14.19 ± 2.02	4	0.32 ± 0.14	4	2.20 ± 0.64	4	1.13 ± 0.37	4	7.90 ± 1.45
p		0.002		0.02		NS		NS		NS
28 days' gestation										
Control	20	28.62 ± 4.58	12	0.65 ± 0.15	12	2.23 ± 0.34	12	2.25 ± 0.62	12	7.81 ± 1.09
Amniotic fistula	15	23.74 ± 5.48	14	0.48 ± 0.13	14	1.99 ± 0.26	14	2.00 ± 0.47	14	8.43 ± 1.05
p		0.007		0.005		0.02		NS		NS

*Mean values ± SD.
†Number assessed.

have only fetal deaths at reexploration. Peritonitis was identified in six does, occurring in 75% (three of four) of those aborting and 60% (three of five) of those having only fetal deaths. Overall, 54 of 160 fetuses (35%) survived until planned cesarean delivery on either day 26 or 28 of gestation. If the does who aborted or had no viable fetuses are discounted, 54 of 88 fetuses (61%) survived until planned delivery. Seven of 14 instrumented fetuses (50%) and 12 of 19 control littermates (63%) were viable at delivery on day 26. Similarly, 15 of 27 instrumented (56%) and 20 of 28 controls (71%) survived until delivery at 28 days (Fig. 1). The continued patency of the amniotic fluid fistula was confirmed by the finding of little or no amniotic fluid when viable instrumented fetuses were delivered.

Amniotomy and maintenance of the amniotic fluid fistula produced significantly smaller fetuses with smaller lungs than those of controls at both 26 and 28 days of gestation (Table I). The lungs were smaller not only by absolute weight but also by lung weight/body weight ratio at 28 days of gestation. No differences in liver weight or liver weight/body weight ratio were discovered on analysis of instrumented and control fetuses at either gestational age. Based on gross inspection, instrumented fetuses appeared to have decreased subcutaneous fat and more translucent skin compared to control fetuses. Three of 22 viable instrumented fetuses (14%) showed evidence of oligohydramnios deformation syndrome. Two fetuses exhibited positional abnormalities of the limbs, and one had mild facial asymmetry.

The maximum lung volume at a transpulmonary pressure of 35 cm of water for both instrumented and control fetuses was the same at 26 days. By 28 days, instrumented fetuses tended to have a smaller lung volume than control fetuses but this difference was not statistically significant (Fig. 2). Standardization of maximum lung volume to fetal body weight revealed no difference at either 26 or 28 days of gestation (Fig. 3). Comparison of maximal lung volume to lung weight at 28 days also failed to show a difference (Fig. 4).

The lung volumes expressed as percentages of maximum lung volume at a transpulmonary pressure of 35 cm of water during inflation were essentially identical for instrumented and control fetuses at both 26 and 28 days. With the mother considered as the random factor, two-factor analysis of variance of the deflation limb revealed no difference at 26 days (Fig. 5). However, percentages of maximum lung volume at a transpulmonary pressure of 35 cm of water during deflation at pressures of 10 cm and 15 cm of water were significantly larger for instrumented fetuses compared to control fetuses at 28 days. Additionally, at a pressure of 0 cm of water there was a tendency for greater residual lung volume (Fig. 6).

Comment

We chose the rabbit as our animal model because of its surgical practicality, multifetal gestation, and the extent of reported data on fetal growth and development available for comparison. The fetal rabbit lung develops through the same stages as the human. The glandular stage is completed at day 24 (human, day 112) with the canalicular stage occurring from day 24 to 27 (human, day 112 to 168). The final intrauterine stage, saccular, takes place between day 27 and term, which for the rabbit is 31 days (human, 280 days). Therefore our study design created chronic amniotic fluid leakage resulting in oligohydramnios extending from late glandular through canalicular into the saccular stage of development. This duration of oligohydramnios should result in pulmonary hypoplasia characterized by inadequate development of pulmonary acini and restricted vascularization. These events would normally

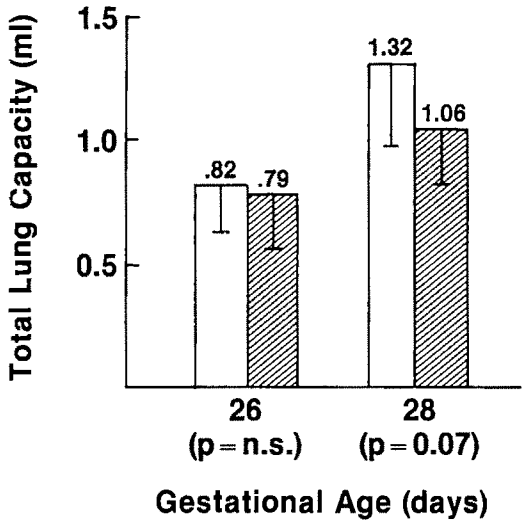


Fig. 2. The maximum lung volume for control *(open bar)* and instrumented *(hatched bar)* fetuses at 26 and 28 days of gestation.

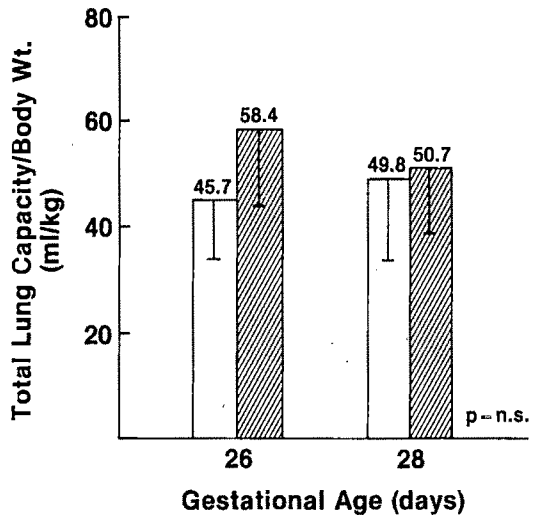


Fig. 3. The maximum lung volume expressed per kilogram of fetal body weight for control and instrumented fetuses at 26 and 28 days of gestation.

occur during the canalicular stage of development. Human fetuses and neonates with pulmonary hypoplasia from congenital hydronephrosis, prolonged premature rupture of membranes, or diaphragmatic hernia often have pulmonary histologic changes compatible with developmental arrest at 20 weeks' gestation or earlier.[7]

Our studies support the theory that the compressive effects of oligohydramnios inhibit normal fetal body and lung growth. Nakayama et al.[12] have shown similar effects in rabbit fetuses exposed to oligohydramnios from day 24 until term. Other investigators have confirmed a detrimental effect on lung weight from reduction of thoracic volume by either creation of a diaphragmatic hernia[13] or insertion of intrathoracic balloons.[14] Fetal breathing and intrapulmonary fluid are thought to be necessary for normal lung development.[10] While body weight, lung weight, and lung weight/body weight ratio were affected by oligohydramnios, liver weight and liver weight/body weight ratio were unchanged. This finding suggests that umbilical blood flow was not substantially impaired and that uteroplacental insufficiency does not occur following premature rupture of membranes. Wesson et al.[15] have shown that ligation of the fetal rabbit trachea will result in decreased body weight similar to that found in neonates with esophageal or duodenal atresia. Oligohydramnios caused by chronic leakage may deprive the fetus of normal amniotic fluid swallowing and its possible influence on fetal protein metabolism or mucosal enzyme maturation. We recognize that this model does not exactly reproduce the occurrence of premature rupture of membranes in the human. A state of continuous anhydramnios was created by the fistula in contrast to the intermittent loss usually associated with human premature rupture of membranes.

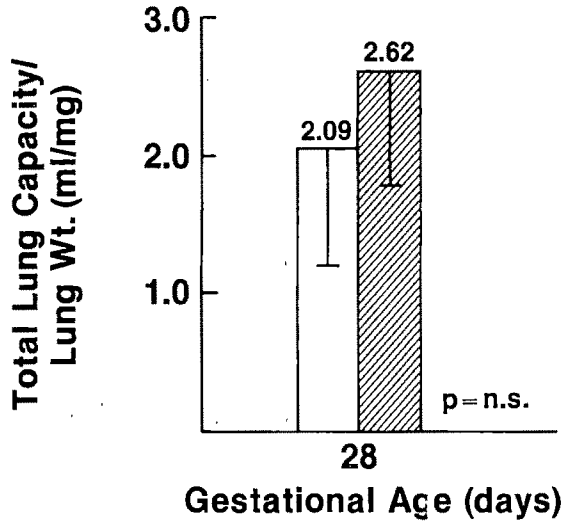


Fig. 4. The maximum lung volume expressed per milligram of wet lung weight at 28 days' gestation.

The pattern of lung weight/body weight ratio for control fetuses is similar to that reported previously[16] with the highest ratio at day 26 and a subsequent decrease at day 28. In our study the ratio followed the normal curve but exhibited a significant difference for instrumented fetuses at day 28. Our belief is that the stress of oligohydramnios has resulted in a stimulus for lung differentiation and depletion of glycogen stores at a significantly faster rate than would normally occur. It has been shown that fetal weight is dependent on uterine horn implantation site.[17] We attempted to negate implantation site bias by instrumenting alternate gestational sacs rather than all pregnancies implanted in a particular uterine horn. We did not correct weight

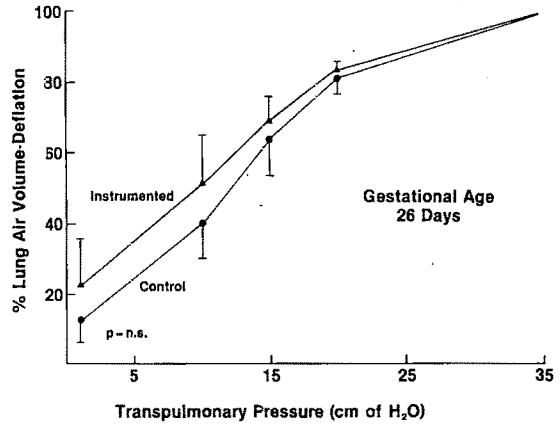


Fig. 5. The proportionate lung volumes expressed as a percentage of total lung volume tended to be higher for instrumented fetuses at 26 days, but these differences were not statistically significant. Mean ± 1 standard deviation shown.

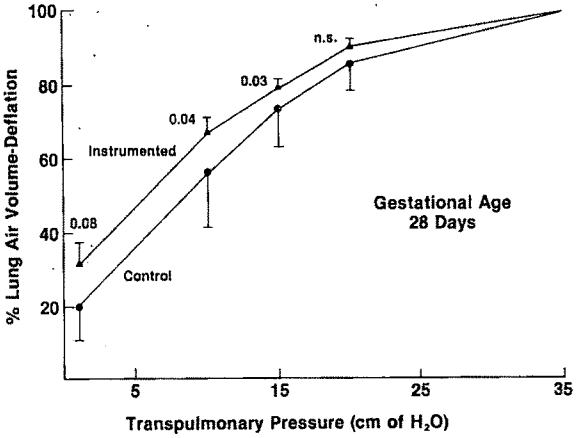


Fig. 6. The proportionate lung volumes at 28 days revealed significantly increased air trapping for instrumented fetuses at 10 cm and 15 cm of water. Mean ± 1 standard deviation shown.

for implantation site, and we assumed random fetal loss at all implantation sites.

We were concerned about the high fetal loss rate. However, we found that as our experience with animal handling, anesthesia, and surgical manipulation improved, our loss rate decreased. Maternal weight loss in the postoperative period did not correlate with fetal loss. Peritonitis developed at random in spite of antibiotic prophylaxis, but when it occurred there was 100% fetal loss. Tocolytic agents were not employed to prevent preterm labor or abortion.

Total lung volume was unchanged at 26 days and showed only a trend toward a decreased lung capacity at 28 days. If thoracic compression alone inhibits development of the acini, then a decrease in lung volume would be expected. Standardization of total lung volume to body weight showed no differences at either gestational age. Since the lung weight/body weight ratio was significantly different at 28 days, the relationship of maximum lung volume to wet lung weight was evaluated. No statistically significant differences were noted; however, the calculated value for instrumented fetuses was higher. One possible explanation is more efficient ventilation of a normal number of acini resulting from a decrease in pulmonary glycogen.

The changes noted during the deflation limb of the pressure/volume studies were the most important findings of our study. There was a trend toward more stable lungs for instrumented fetuses at 26 days, but the differences were not statistically significant. However, at 28 days there was significantly more air trapping in the lungs of instrumented fetuses. These findings indicate not only more stability but also more functional maturity. The exact cause of the improved stability and maturity is unknown. It has been postulated that there is both a decrease in lung connective tissue and an increase in alveolar surfactant.[11] The rate of fetal lung maturation has been shown to be dependent on fetal sex, with females approximately 1.5 days ahead of males.[17] Because of our small numbers, we were unable to separately analyze male and female outcomes.

The absolute risks and benefits of prolonged premature rupture of membranes remain unknown. We have shown both detrimental (weight alterations) and beneficial (improved lung stability) effects of oligohydramnios. It has been reported that the effects of oligohydramnios on body weight and lung weight can be reversed by relieving thoracic compression by infusion of fluid into the uterine cavity.[12] The potential effect of this approach on pressure/volume studies will be examined in the future. Currently we are fractionating lung phospholipids and examining both light and electron microscopic lung sections. We plan to continue our study on a larger basis to examine the effects of fetal sex and uterine implantation site. In addition, we would like to explore the issue of a critical day for the development of pulmonary hypoplasia related to amniotic fluid loss.

We gratefully acknowledge the help of Dr. Susan Ahmed with statistical analysis, Ruth Anne King with surgical assistance, and Richard Rabold with performance of the pressure/volume studies.

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Discussion

Dr. Bruce K. Young, New York, New York. Studies of the growth and development of the lung offer insight into the pathogenesis of many important syndromes in perinatal medicine. There are four stages of human fetal lung development: (1) embryonic, from conception to the fifth week; (2) pseudoglandular, from the fifth to the seventeenth week; (3) canalicular, from the sixteenth to the twenty-fourth week; (4) terminal sac period, from the twenty-fourth week to term. By the sixteenth week of gestation, all of the branches of the tracheobronchial tree up to the terminal bronchioles are established, and new branches cannot be formed thereafter. Thus the preacinar airways cannot be increased after this time, and only the gas-exchanging portion, composed of the respiratory bronchioles, alveoli, and alveolar ducts, will differentiate after about 16 to 20 weeks of gestation. Therefore an insult to lung development during fetal life will have different pathologic effects, depending on when it occurs. Further, there are many causes of pulmonary hypoplasia in the human fetus, and their effects are variable, depending on the time of onset and duration during fetal life. These include renal agenesis and dysplasia, urethral obstruction, phrenic nerve agenesis, thoracic dystrophy, diaphragmatic hernia, Rh isoimmunization, polyhydramnios, and premature rupture of membranes, the basis for the present study.[1-3]

Premature rupture of membranes of the human fetus associated with prolonged oligohydramnios has been shown to produce pulmonary hypoplasia and anatomic deformities, particularly when present before the twenty-sixth week of gestation, and when the duration of rupture is >5 weeks.[3,4] Thus, an animal model would be very helpful in determining the pathophysiology, and in formulating any possible plan of therapy. Dr. King and his associates chose a well-described model, the rabbit with amniotic fistula, to study the effects of oligohydramnios on fetal growth and lung development.[5,6] Their data demonstrate statistically significant reductions in birth weight and lung weight in the fetuses subjected to oligohydramnios compared to the control fetuses. This was true for both 26-day and 28-day animals. The ratio of lung weight to birth weight, an index of specific pulmonary hypoplasia, was lower in the 28-day animals with amniotic shunt, with statistical difference at the 0.02 level. Another observation of interest was the 14% incidence of deformation syndrome in the rabbit fetuses with amniotic shunting. Finally, the lung volume studies revealed no difference in maximum lung volume or the ratio of maximum lung volume to body weight. However, in the 28-day animals the percentage of maximum lung volume at deflation with use of pressures of 10 cm and 15 cm of water was greater for shunted lungs, indicating air trapping. The authors state that this indicates not only more stability but more functional maturity. These very interesting observations lead to a number of specific questions.

1. Did the authors make any measurements of amniotic fluid in the shunted and control animals?

2. Since there is a relationship between implantation site and weight, did the authors consider using the contralateral horn fetuses as controls for this confounding variable?

3. At 26 and 28 days there was no difference in lung volume between the shunted and control animals. Since it is recognized that inhibition of fetal breathing by cord transection will induce lung hypoplasia, and this is preventable by tracheal ligation in the rabbit, would the authors consider that a lower intrapulmonary pressure might be more important than compressive effects, since one would expect that compression would reduce the lung volume[7,8]?

4. Why was air trapping seen at pressures 10 and 15 cm of water, but not at the other transpulmonary pressures studied?

5. Would the authors agree that the changes observed during the deflation studies are also consistent with decreased lung connective tissue recoil, as well as with hyperinflation air trapping, as seen with erythroblastosis, until their histologic and biochemical evidence of lung maturity is forthcoming?

Dr. King and his colleagues have presented important work. Oligohydramnios is associated with abnormal pulmonary growth and development in a variety of clinical conditions, for which we have no standard treatments. The authors have addressed the pathophysiologic conditions in a careful study, which has given us the glimmerings of understanding the clinical problem. Further studies addressing the anatomy, histology, and biochemistry of the lungs in this animal model are likely to elucidate the role of oligohydramnios in pulmonary hypoplasia.

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DR. CHARLES E. FLOWERS, Birmingham, Alabama. I would like to point out two things.

There is a tremendous reduction in the volume of amniotic fluid during the last 3 days of rabbit gestation. Therefore one must be careful to study through appropriate transmission electron microscopy and enzymatic studies the growth of lung tissue in the shunted animals and the normal rabbits during the twenty-seventh to the thirtieth day of normal gestation.

Second, you have adversely affected uterine blood flow by putting in these uterine shunts. It therefore becomes very important to compare the pups in the contralateral horn, since the shunts can affect uterine blood flow and pulmonary growth.

DR. CHARLES A. WHITE, New Orleans, Louisiana. Dr. King has presented some interesting observations that I presume many of us would like to eventually translate to the solution of clinical problems involving the infants in cases of protracted periods of ruptured membranes.

We do on occasion see these cases and are concerned about the so-called "stiff lung" or the reduced ability of the neonates to adjust to extrauterine respiratory efforts.

Now that Dr. King and his colleagues have established this animal model, do you see merit in extending your studies to allow these treated fetuses to attempt an extrauterine life, allowing them to be delivered either by section-hysterotomy or by natural birth to determine their ability to establish ventilatory efforts as compared to the control animals? That might give us more comparative data to assist in these problems in humans.

DR. KING (closing). Dr. Young's first question was, Did the authors make any measurements of amniotic fluid volume in the shunted and control animals?

No, we did not do that. This had been done previously by Nakayama et al., whose model we patterned ours after, and they could show a statistically significant difference with no fluid in the instrumented gestation and normal amniotic fluid volume in the control gestations.

We did not feel it was necessary to repeat that phase of their study.

The next question was in relationship between implantation site and weight. Did the authors consider using contralateral horn fetuses?

Again, we patterned our studies after Nakayama et al., who did use the contralateral horn. During the preliminary phase of this study I found that the gestations were not equally distributed for some reason. Therefore, to try to eliminate the bias of having only two gestations on one horn and seven or eight on the other, we decided we would alternate our instrumented and controlled gestations.

We realize that this may exert some compression of amniotic fluid or uteroplacental blood flow in the adjacent gestational sacs, but we felt that this would eliminate the bias of horn implantation site.

The next question had to do with tracheal ligation and lower intrapulmonary pressure. Those are true comments, and it has indeed been shown that intrapulmonary pressure is very important. There have been some studies in the past in which tracheal fluid has been continually aspirated from the trachea and pulmonary hypoplasia has been demonstrated.

The next question was, Why was air trapping seen at pressures of 10 and 15 cm of water and not at the other transpulmonary pressure studies?

Our thought is that it probably will be seen with continuing gestation and although we could show no difference at 26 days, by 28 days at 15 and 10 cm there was statistically significant difference, and at 0 cm there was an elevation though not statistically significant. Our feelings are that if this model is continued to 30 or 31 days, we may see a further separation between the instrumented gestations and the control ones.

Would the authors agree that the changes observed during deflation studies were also consistent with decreased lung tissue recoil? Yes, I think that is one of our primary theories.

I can give you some preliminary results on the biochemical and electron microscopic examination.

We could find no difference in total phospholipids in the instrumented animals compared to the control

ones. We are currently fractionating those phospholipids, so we will have some additional data in the future.

With regard to the electron microscopic examination, there is no question that the appearance of the lamellar bodies in the type II alveolar cells in the twenty-sixth day and twenty-eighth day animals were more numerous in the instrumented animals. In addition, the lamellar body appears to be a denser lamellar body at the twenty-sixth day after only 3 days of induced oligohydramnios. At this point we do not have a way to quantify the amount of this production, but we will do that in the future.

Regarding Dr. Flower's question about the last three days of gestation, we would agree that continuation through term and even continuing on, as has been suggested by Dr. White, would be important to learn how these animals tolerate labor, delivery, and early newborn existence. We plan to continue with these studies and will investigate those problems.

Temporal and receptor correlates of the estrogen response in sheep

Frank C. Greiss, Jr., M.D., James C. Rose, Ph.D., Timothy E. Kute, Ph.D., Randall T. Kelly, M.D., and Lisa S. Winkler, B.S.

Winston-Salem, North Carolina

Uterine blood flow and uterine cytosol and nuclear estrogen receptors were measured at critical times during estradiol-induced vasodilatation in acute anesthetized and chronic conscious sheep preparations at estradiol bolus injection frequencies from 1 to 24 hours. During acute experiments, the uterine blood flow response was muted and cytosol estrogen receptor replenishment did not occur whereas full replenishment occurred in 12 hours in conscious ewes. In ewes treated with daily estradiol that had a stable daily uterine blood flow response, the uterine blood flow response 24 hours after uterine biopsy was similar to the preoperative one. Analysis of the duration of peak uterine blood flow levels and the rate of uterine blood flow descent from peak levels showed that an interval of 18 hours between estradiol injections was necessary for the uterine blood flow response to approximate that observed after 24 hours. These observations suggest: (1) that uterine vascular receptor replenishment is delayed compared with that of the total uterus; (2) that operative stress compromises cytosol estrogen receptor metabolism and possibly nuclear estrogen receptor function; (3) that the delayed maximum uterine blood flow response to estradiol in ewes previously untreated with estradiol is due to a trophic uterine effect of daily estradiol stimulation. (AM J OBSTET GYNECOL 1986;154:831-8.)

Key words: Uterine blood flow, estrogen, estrogen receptors, uterine vasodilatation

The uterine blood flow response to estrogenic stimulation has been studied extensively in the oophorectomized ewe. When estradiol-17β is injected intravascularly, uterine blood flow is unchanged for 30 minutes. Then a progressive marked increase occurs and reaches peak levels after 1.5 to 2.0 hours. Thereafter, uterine blood flow gradually declines to control levels during 12 to 18 hours.[1] This response can be duplicated daily. Dose-response curves indicate that the maximum response occurs from 1 μg/kg given intravenously, 0.5 μg/kg intra-arterially, or 0.5 to 1.0 μg given directly into the uterine artery.[2, 3] If the estradiol injection is given during a continuous infusion of cycloheximide into the uterine artery, the response is ablated.[1] This observation and the latent phase before the increase in uterine blood flow have been interpreted to indicate that estradiol induces the formation of a vasodilator substance that mediates the vascular response. Although substances have been identified that can cause a response quantitatively similar to that of estradiol, none of these has been shown to be the mediator per se. One other characteristic of the uterine blood flow response is pertinent. At ovine estrus, uterine blood flow increases from very low preestrous levels to about half of those observed after maximum doses of estradiol, and these levels are maintained for 24 hours or more.[4] Presumably, the uterine blood flow rise is caused by and lags about 6 hours behind a similar curve of estradiol secretion. However, in our laboratory and in experiments reported to date, a sustained increase in uterine blood flow has never been induced by exogenous estradiol administration except when the estradiol is given intramuscularly in oil. In fact, when estradiol is infused continuously at maximum pharmacologic doses, the uterine blood flow response is no different from that after a single injection.[2] These observations suggest that when maximum pharmacologic doses of estradiol are given, a refractory phase occurs, which may be related to the metabolism of uterine estrogen receptors. To test this hypothesis, we have measured cytosol and nuclear receptors in serial uterine biopsy specimens obtained at critical times during the uterine blood flow response to estradiol in acute and chronic experiments in sheep. In addition, further knowledge of the refractory period has been achieved in conscious ewes by observation of the uterine blood flow response to various frequencies of intermittent estradiol injections.

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Methods

Animal preparations

Group 1. In a preliminary group of two ewes anesthetized with pentobarbital sodium, biopsy specimens were obtained from the upper, middle, and lower thirds of the uterine horn and the animals were put to death. The biopsy specimens were analyzed for cytosol estrogen receptor content.

Group 2. Nonpregnant multiparous ewes were anesthetized with intravenous ketamine and supplemental pentobarbital sodium when necessary. Bilateral oophorectomy, implantation of an electromagnetic flow probe around the left middle uterine artery, and implantation of an aortic occlusion back loop were performed as previously described.[5, 6] Two catheters were threaded via the mammary artery and vein into the descending aorta and inferior vena cava for blood pressure monitoring and estradiol injections. After the ewes recovered from operation, daily intravenous bolus injections of estradiol, 1 μg/kg, prepared after the method of Killam et al.[1] were given and uterine blood flow was monitored until similar daily flow peaks occurred. Then a uterine biopsy specimen from the horn contralateral to the flow probe was obtained at laparotomy with the animal under ketamine anesthesia 24 hours after the previous estradiol injection. On recovery, daily estradiol doses were continued for 4 to 7 days. Then repeat uterine biopsy specimens were obtained in different animals 6, 12, and 18 hours after the last estradiol injection. Then the ewes were put to death. In a subset of this group biopsy specimens were obtained 24 hours after a previous biopsy and estradiol injection.

Uterine blood flow responses were analyzed as in group 4 described below and uterine biopsy specimens were analyzed for cytosol estrogen receptor and compared by means of analysis of variance and two-tailed Student's *t* test.

Group 3. Ewes were prepared as in group 2 and after repetitive daily estradiol-induced uterine blood flow peaks occurred, the following acute experiments were performed 24 to 27 hours after the preceding estradiol injection.

Ewes were paralyzed with curare, intubated while under topical anesthesia, and anesthetized with a 2:1 mixture of nitrous oxide–oxygen. The rate and frequency of respirations were adjusted by a Harvard small animal respirator to maintain normal pH and blood gas levels. Supplemental curare was used as needed throughout the experiment. When uterine blood flow and maternal blood pressure were stable, the uterus was exposed via a midline incision and a control biopsy specimen (1 to 2 gm) from the uterine horn contralateral to the flow probe was obtained. Then an intravenous bolus of estradiol, 1 μg/kg, was given. Repeat uterine biopsy specimens were obtained 5 and 30 minutes and 2, 6, 10, 14, and 18 hours later and the ewes were put to death. Between biopsies, the abdomen was closed to minimize trauma. Biopsy specimens were analyzed for cytosol estrogen receptor and nuclear estrogen receptors as described below. Since cytosol estrogen receptor depletions were similar to those observed in conscious ewes (group 2), control experiments to exclude an effect of operative stress were not performed.

Group 4. In a final group of ewes chronically prepared as in group 2, after daily estradiol-induced uterine blood flow peaks occurred, uterine blood flow responses were observed after the following regimens of intravenous estradiol, 1 μg/kg, bolus injections: every hour for eight doses, every 2 hours for five doses, every 3 hours for four doses, every 4 hours for three doses, and every 6 hours for three doses. Further responses were observed after estradiol given 8, 10, 12, 14, 16, and 18 hours after the preceding estradiol dose. The various estradiol administration regimens were randomized so as to minimize the possible effect of time from initial operation on the observed responses. Five 1-minute uterine blood flow levels were averaged every half hour during response periods and results were expressed as a percentage of the maximum flow observed during each individual response. Intraresponse levels at the various dose frequencies were compared after 4 hours with those of the 24-hour dose frequency by means of analysis of variance and the Mann-Whitney test for multiple comparisons.

Cytosol receptor assay

Preparation of cytosol. The cytosol fractions were prepared by the method of Anderson et al.[7] All procedures were done at 4° C unless otherwise stated. Approximately 0.5 gm of frozen tissue was weighed out and minced into small fragments with a scalpel. The minced tissue was put in a Kontes glass grinder along with 2 ml of buffer A (10 mmol/L Tris, 1.5 mmol/L ethylenediaminetetraacetic acid, 10% glycerol, and 1.0 mmol/L monothioglycerol, pH 7.4 to 7.5) and homogenized. After the homogenate was transferred to a centrifuge tube, another 4 ml of buffer was added. The suspension was centrifuged at 1000 × g for 10 minutes to separate the nuclear and cytosolic fractions. The cytosolic fraction was mixed on a vortex mixer with a dextran-coated charcoal pellet (6 ml of 1.0% Norit A and 0.5% dextran) to remove endogenous estradiol and incubated for 10 minutes at 0° C. After the incubation period, the suspension was centrifuged at 120,000 × g for 30 minutes. The supernatant protein was measured by the Waddell method and normalized to 2 mg/ml for the binding study.

Cytosolic binding assay. The cytosol fraction was used in binding studies done by Scatchard analysis[8] and the assay of Katzenellenbogen et al.[9] The Scatchard analysis uses 10 points with doses of tritiated estradiol varying

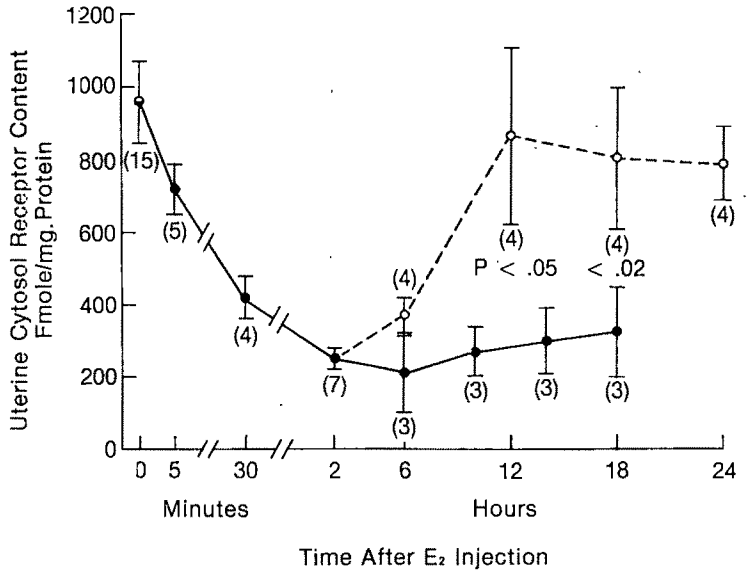


Fig. 1. Uterine cytosol estrogen receptor content (mean ± SEM) after intravenous estradiol injection (1 μg/kg) in continuously anesthetized (●) and previously conscious (○) ewes. *Numbers in parentheses* indicate number of observations.

from 0.1 to 10 nmol ± 5×10^{-7} mol/L DES. The addition of DES is to correct for nonspecific binding. In the case of Scatchard analysis, the specific binding is defined as the total binding of receptors minus the nonspecific binding of receptors. The reactions were stopped with dextran-coated charcoal and analyzed by a computer program.[10]

The assay of Katzenellenbogen et al., which determined total binding in the cytosol, uses triplicates of 10 nmol/L tritiated estradiol ± 10×10^{-7} mol/L DES. Cytosol was added to each vial and incubated for 1 hour at 0° C, then at 30° C for 18 hours. The reactions were then cooled to 0° C and stopped with dextran-coated charcoal. The calculations for the specific binding in this case were based on total binding minus nonspecific binding divided by the total protein concentration and reaction volume.

Nuclear binding assay. The nuclear fraction was assayed by the method described by Anderson et at.[7] The nuclear suspension was vigorously mixed on a vortex and 0.25 ml aliquots were transferred into two tubes containing 13 nmol/L tritiated estradiol and two tubes containing 13 nmol/L tritiated estradiol + 13×10^{-7} mol/L estradiol. To each tube 0.25 ml of buffer was added and mixed on a vortex. These reactions were incubated in a 37° C shaking warm water bath for 1 hour. Then to remove any free steroid the reactions were washed three times with 1 ml of buffer A and centrifuged at 1000 × g for 10 minutes. The supernatant was discarded and the nuclear pellet resuspended in 1.0 ml of ethanol and incubated for 30 minutes at 30° C. Aliquots of 0.3 ml were taken from each tube and counted. The specific nuclear binding activity was calculated in femtomoles per gram of tissue and femtomoles per milligram of deoxyribonucleic acid (DNA).[11]

Table I. Uterine cystosol estrogen distributions*

	Ewe No.	
Horn location	*81-201*	*81-202*
Upper third	637	1523
Middle third	545	1664
Lower third	721	1865

*Femtomoles per milligram of protein.

Results

Estrogen receptor responses. Cytosol estrogen receptor content in three different parts of the uterine horn were determined in two control ewes (group 1). These distributions are shown in Table I and indicate that receptor content in different areas of the ovine uterus is similar.

Uterine cytosol estrogen receptor responses were measured by analysis of specimens from timed biopsies during 18 hours in five anesthetized ewes (group 3). Complete results were obtained in only three ewes. Within 30 minutes of the estradiol bolus injection, cytosol estrogen receptor receptors decreased 57% from control, reached a nadir 75% below control levels after 2 hours, and stayed at this level for the remainder of the observation time (Fig. 1, *closed circles*).

The time course of cytosol estrogen receptor replenishment in 10 ewes 2 to 10 days after prior operation and 6, 12, and 18 hours after estradiol injection are

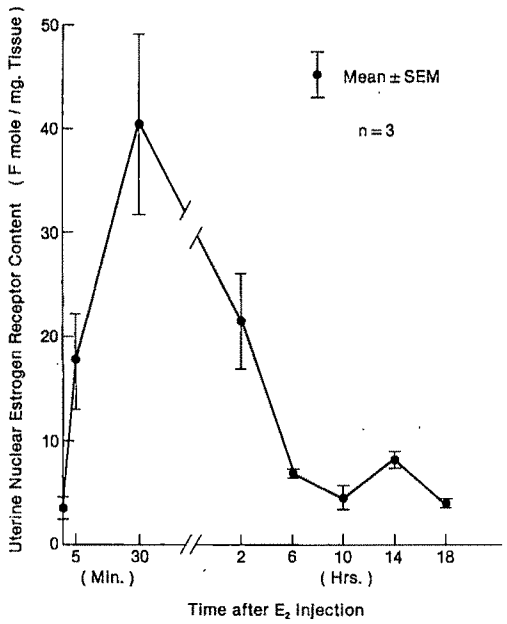


Fig. 2. Uterine nuclear estrogen receptor content after intravenous estradiol injection (1 μg/kg) in continuously anesthetized ewes.

also shown in Fig. 1 *(open circles)*. The four assays at 24 hours were obtained 24 hours after a previous biopsy and estradiol injection.

Uterine nuclear estrogen receptor responses during 18 hours in three of the anesthetized ewes (group 3) are shown in Fig. 2. The nuclear estrogen receptor levels peaked 30 minutes after the estradiol injection and rapidly decreased to control levels within 6 hours.

Uterine blood flow responses. In four of the five ewes monitored before and during uterine biopsies while under anesthesia (group 3), the typical uterine blood flow response after estradiol occurred. However, the magnitude of the response was markedly reduced and achieved less than 50% of that evoked 24 hours earlier in the conscious state. In the one ewe in which no uterine blood flow response occurred, the cytosol estrogen receptor responses were similar to those in the other ewes.

During daily recordings of uterine blood flow responses before and 24 hours after uterine biopsies in ewes in group 2, it was noted that peak uterine blood flow levels were approximately the same as those observed during the days preceding the biopsy. These are shown in Table II.

Eighty-six regimens of bolus doses of estradiol at varying hourly frequencies were administered to nine ewes. Composite uterine blood flow responses at 1-, 2-, 3-, 4-, and 6-hour dose frequencies are illustrated in Fig. 3. *Panel 1* shows the uterine blood flow response during hourly estradiol doses during 8 hours and the control response after 24 hours. Except for a more

Table II. Postoperative uterine blood flow responses to estradiol after preoperative estradiol stabilization

Ewe No.	*Range of preoperative responses**	*24 h postoperative response**
84-109	80-105	83
84-110	89-100	89
	89-100	92
84-100	83-107	94
	83-107	86

*Maximum uterine blood flow (milliliters per minute).

gradual descent of uterine blood flow from peak levels, the hourly dose response was similar to the 24-hour one. When estradiol was administered every 2 hours (*panel 2*, Fig. 3), the maximum response was reachieved 1 hour after the second dose but further doses only muted the waning response or caused a minimal uterine blood flow increase. Administrations every 3 hours (*panel 3*, Fig. 3) caused muted uterine blood flow increases 1 to 1½ hours later but failed to alter the progressive descent from the maximum uterine blood flow levels induced by the first dose. When estradiol was administered every 4 hours (*panel 4*, Fig. 3), peak flow rates recurred transiently after the second dose but only a muted rise occurred after the third dose. It was not until estradiol was administered every 6 hours (*panel 5*, Fig. 3) that uterine blood flow peaks equal to those induced by the first dose could be reinduced regularly. However, the duration of the peak responses was shorter and the rate of uterine blood flow descent from peak levels was more rapid than after the first dose.

The uterine blood flow rates after estradiol injections timed 6, 8, 10, 12, 14, 16, 18, and 24 hours after the previous injection are illustrated in Fig. 4. As the estradiol dose interval increased, the rate of uterine blood flow descent from peak levels became slower and the sustained uterine blood flow rate between 1.5 and 2 hours reappeared. At the 18-hour dose frequency, although the uterine blood flow rates after 4 hours were slightly lower than the 24-hour ones, they were not significantly different whereas those at dose frequencies between 6 and 16 hours were different ($p < 0.02$).

Comment

Current steroid response theory indicates that a given response is dependent on the quantity of steroid exposed to a cell and the concentration of steroid-specific macromolecule receptors present in the cytosol of target cells. Upon formation of the steroid-receptor complex, it is translocated to the cell nucleus (nuclear receptor) where transcriptional events begin the process of effecting the various actions of the steroid. Since the initial concentration of cytosol receptor controls the

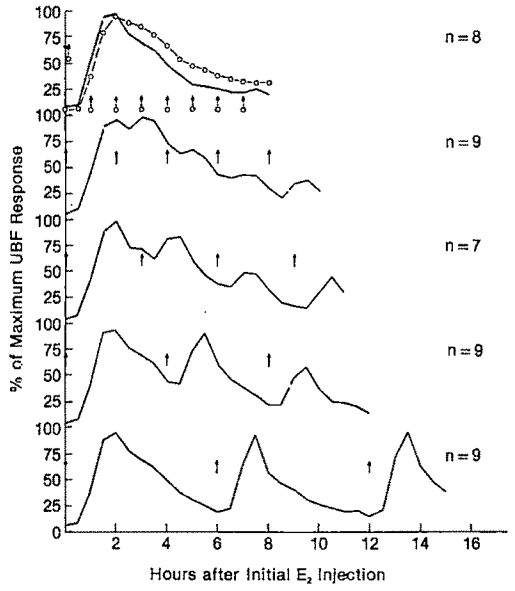


Fig. 3. Uterine blood flow responses after intravenous estradiol injections (1 μg/kg) (↑) at different injection frequencies. *Upper panel* compares 24-hour dose frequency *(solid line)* with hourly dose frequencies (♂ and o——o).

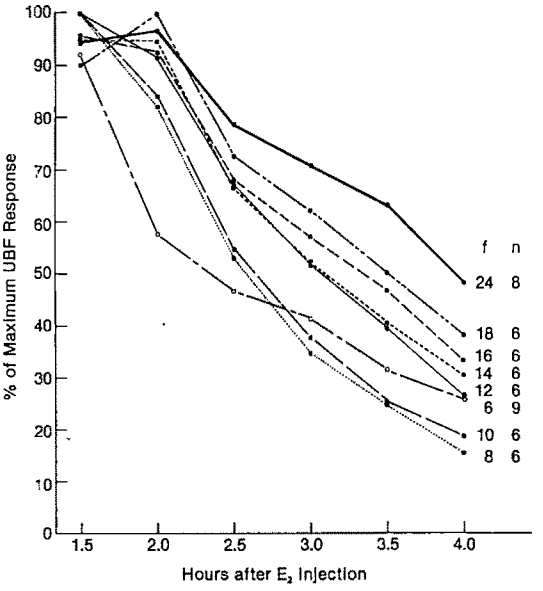


Fig. 4. Uterine blood flow responses after intravenous estradiol injections (1 μg/kg) at dose frequencies *(f)* between 6 and 24 hours. All of the responses at 4.0 hours are different from the 24-hour response ($p < 0.02$) except that after 18 hours.

nuclear receptor, a depletion of cytosol receptor will occur with translocation and the process of cytosol receptor replenishment must occur. Until a sufficient amount of cytosol receptor is replenished, the steroid-induced response will be blunted or erased depending on the degree of cytosol receptor depletion induced by the dose of administered steroid. This highly simplified description of a complex process provides the basis for using a continuously monitorable response to estradiol stimulation, uterine vasodilatation, to identify critical times for uterine biopsies for cytosol estrogen receptor assay and possibly to permit the response itself to give insight into cytosol estrogen receptor replenishment. We appreciated at the outset that cytosol estrogen receptor and nuclear estrogen receptor responses of samples of entire uterine tissues may not reflect those of the uterine vasculature but felt that such responses in the light of uterine blood flow changes may complement interpretation of responses. Since cytosol estrogen receptor contents were similar in different parts of the uterine horn, we felt that multiple biopsies from differing parts of the horn provided valid data for comparison.

It is clear from Fig. 1 that the intravenous dose of estradiol necessary to evoke maximal uterine vasodilatation uses only 75% of available total uterine cytosol estrogen receptor. The uterine blood flow responses during injections of estradiol every hour and every 2 hours (Fig. 3) suggest that similar incomplete depletions in the cytosol estrogen receptor of the uterine vasculature occur; that is, since the period of maximal dilatation can be prolonged or regained during the time of maximal initial depletion of cytosol estrogen receptor, it is likely that such responses are mediated by residual cytosol estrogen receptor. It would follow that recurrent uterine blood flow elevations after estradiol injections given 3, 4, and 6 hours after the initial injection would reflect the sum of residual and replenished cytosol estrogen receptor. In conscious unstressed ewes, cytosol estrogen receptor replenishment occurred within 12 hours of estradiol administration. These results are consistent with those reported in rat uteri by Clark and Peck.[12] However, the uterine blood flow responses suggest that cytosol estrogen receptor replenishment in the uterine vasculature proceeds at a slower pace than that in the uterus as a whole. For example, repetitive uterine blood flow peak flows could not be induced by estradiol until 6 hours after the preceding estradiol injection, a time when total uterine cytosol estrogen receptor replenishment was 39% of control. However, these peak flows were dissimilar from 24-hour controls in that the duration of the response was much shorter. This led us to look at the vasodilatory response from two aspects, its absolute magnitude and its magnitude over time. Methodologic limitations dictated the 4-hour observation period. Since duplication of uterine blood flow levels observed 24 hours after a preceding estradiol injection required an 18-hour interval, we have interpreted this to indicate that vascular cytosol estrogen receptor replenishment sufficient to duplicate control levels requires at least 18 hours. A longer time to full replenishment cannot be

excluded, however, since the control response was achieved presumably with <100% of available cytosol estrogen receptor.

Investigators in this area have observed regularly that in animals under anesthesia, the estradiol vasodilatory response is muted and that after initial oophorectomy and flow probe implantation in ewes previously untreated with estradiol, at least 3 to 4 days of daily injections of estradiol is required to evoke a maximum repetitive uterine blood flow response. Anecdotally, both observations have been attributed to operative stress. This might be a function of operative and postoperative physiologic responses not only of the cardiovascular system but of estrogen receptor function as well. The present observations give possible insight into the explanations for these responses. Clearly, operative stress (anesthesia and operation) exerts immediate effects. In every case, the magnitude of the uterine blood flow response was blunted in comparison with responses in conscious unstressed animals and cytosol estrogen receptor replenishment did not occur (Fig. 1). Alternately uterine nuclear estrogen receptor responses were similar to those previously reported in rats.[12] Since cytosol estrogen receptor replenishment was obviously altered, it is possible that, although receptor translocation occurred normally, posttranslocational events were similarly compromised. Such an interpretation is supported by the observations of Lippe and Szego,[13] who determined that the suppressive effect of actinomycin D on estrogen-induced uterine water imbibition is mediated by adrenocortical hyperactivity.

Twenty-four hours after uterine horn biopsy, cytosol estrogen receptor replenishment was apparently complete and when repetitive daily estradiol responses had been achieved before operation, uterine blood flow responses 24 hours later approximated the preoperative ones (Table II). We believe that uterine horn biopsy is at least as stressful as oophorectomy and implantation of a flow probe on the middle uterine artery. If this is true, then the initial low uterine blood flow levels observed after the latter procedure cannot be attributed to a prolonged effect on uterine estrogen receptor metabolism. Rather it would appear that late uterotropic responses (cellular hypertrophy and hyperplasia) permit a progressively greater uterine blood flow response to daily estradiol stimulation that would appear to maximize after 4 to 7 days.

The results presented herein further clarify events of the estradiol-induced vasodilatory response of the ovine uterus. Since most investigators of the estradiol-induced uterotropic responses address themselves to early and late metabolic and biosynthetic responses, we hoped that a continuously monitorable response might improve our understanding in this area. The observed results are consistent with known steroid-receptor interaction theory but the significance of the observations is only speculative because methodologic limitations preclude actual measurement of cytosol estrogen receptor and nuclear estrogen receptor in the uterine vasculature.

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Discussion

Dr. Edgar L. Makowski, Denver, Colorado. Since the observation by Markee[1] that uterine blood vessels become dilated after the administration of estrogens, many investigators have been interested in the steroid stimulus—uterine response relationship In the current study by Greiss and his associates, this relationship was investigated in nonpregnant, oophorectomized sheep by monitoring uterine blood flow and uterine cytosol and nuclear receptors during estradiol-induced vasodilatation. Changes in uterine blood flow were determined by electromagnetic flow probes, and alterations of uterine receptors (cystosol and nuclear) were interpreted according to the classical "two-step" biochemical model of intracellular hormonal interaction.[2] This model hypothesizes that the steroid binds with a soluble receptor in the cytoplasm of target cells The receptor-hormone complex is then translocated to the nucleus

in a temperature-dependent process and there interacts with the nuclear chromatin. This interaction initiates transcription and new protein is synthesized.

In group 1 of the current study, consisting of two animals, uterine biopsy specimens from various portions of the horn were analyzed for cytosol estrogen receptor content. The distribution of these receptors was somewhat similar within the same animal but very dissimilar between animals (see Table I). The first question to the authors is: Was the large difference between animals due to differences in the phase of the estrous cycle? Further, it is not apparent from the manuscript if the uterine tissue biopsy specimens were controlled for the presence, number, and size of caruncles. With the "two-step" steroid biochemical model a temporal relationship was demonstrated between the type of receptors and the classical physiologic response to estrogen stimulation. During acute experiments, as in group 2, the investigators noted a blunted uterine blood flow response to the administration of estradiol, which confirms previous results of Killam et al.[3] A new dimension was added to this observation in that uterine cytosol receptor content continued at low levels for the duration of exposure to stress.

Both groups 2 and 3 received estradiol for 4 to 7 days before the acute experiments. However, there was a difference in time of uterine blood flow measurement between the groups. In group 2 the uterine blood flow was blunted during acute experiments, and in group 3 uterine blood flow response 24 hours after the experiments approximated the preoperative levels. The authors speculate that the latter observation could have been secondary to a uterotropic response after the estradiol administration. This hypothesis would have been strengthened if they had included some observations on uterine blood flow in group 3 at the time of uterine biopsies or in group 2, 24 hours after uterine biopsies. Another question for the authors is whether they have any evidence that the estrogen affinity of receptors in the vascular wall is similar to that of those located extravascularly in the myoendometrium.

In group 4, the authors confirm and expand the previous observations of Clewell et al.[4] that the uterine response depends on the arterial concentration of the steroid and the duration as well as pattern of exposure to estrogen. Indeed, a continuous infusion of estrogen at high doses can result in tachyphylaxis.

As noted earlier, the observations of Greiss et al. on the changes of uterine receptors after estradiol administration are based on the "two-step" biochemical model. Recently the validity of this model has been challenged by Welshons et al.[5] and King and Greene.[6] In the elegant immunocytochemical studies of King and Greene, monoclonal antibodies to estrogen receptor protein were developed. Use of such antibodies in experiments on estrogen-sensitive tissues from humans and nonhumans demonstrated that specific staining occurred only in the nuclei of all stained cells. Their results indicate that the in vivo estrogen receptors are localized in the nucleus. These findings are consistent with the observations reported for vitamin D and thyroid hormone receptors.[7-9] Presence of receptors in cytosol may therefore result from redistribution after homogenization of the cells. Welshons et al. examined the distribution of unbound estrogen receptors in GH_3 cells obtained from rat pituitary tumors. Cytoplast and nucleoplast fractions were obtained from these cells by enucleation with the use of cytochalasin B. By this method, most of the unbound estrogen receptors were associated with the nuclear fraction and little estrogen binding activity was present in the cytoplast. These data alter somewhat the biochemical model that assigns free estrogen receptors to the cytoplasm. In light of these recent findings, one must be very cautious in interpreting the role of estrogen receptors. If further investigation verifies the newer observations, the "two-step" biochemical model will certainly require reinterpretation.

Finally, the authors should be commended for their continued interest in attempting to define the mechanism(s) responsible for the induction of uterine vasodilatation after estrogen administration.

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DR. NORMAN F. GANT, JR., Dallas, Texas. Dr. Greiss, I wonder if there is not another possible explanation.

I am sure you are familiar with the work of Jim Roberts and the fact that adrenergic receptors are increased or decreased, both α-receptors and β-receptors, based on estrogen versus estrogen-progesterone ratios.

Have you looked at or are you planning to look at adrenergic receptors in uterine or vascular smooth muscle in response to estrogen injection or after the stress of anesthesia?

DR. ARTHUR L. HERBST, Chicago, Illinois. I have a question and a comment about the receptors. As Dr. Makowski mentioned, with immunohistologic techniques, the receptors seem to be primarily in the nu-

cleus. This is true also in human tissues such as endometrial carcinoma and ovarian carcinomas.

Some of the data that we have from our institution would suggest, however, that although they are nuclear in location, their quantitation parallels the biochemical measurement. So, when you speak of "cytosol" receptors, you are probably speaking of loosely bound nuclear receptors. Thus the theory of the steroid-receptor interaction eventually leading to ribonucleic acid–mediated protein synthesis would probably still be valid although the name cytosol is probably not valid.

I just would be interested to know if you have done any immunohistologic studies and if the location of the receptor is also nuclear in your preparation?

Dr. Greiss (Closing). For one of my articles, three discussants is somewhat of a record. In particular, I want to thank Dr. Makowski who, in his usual incisive and complete manner, criticized the paper from exactly the same areas I would have myself.

Dr. Makowski, with respect to the variation in the concentration of estradiol receptors, we did not know where the two animals selected were in the cycle. This was unimportant since we were just trying to see whether distributions were similar. However, in the subsequent experiments, all of the ewes were oophorectomized and had been receiving daily estrogen. Despite this there remained very large baseline variations in estradiol receptor concentrations that I am at a loss to explain. These variations, however, account for the wide variations you have seen in the data.

We made no attempt to evaluate receptor content in the caruncles alone.

With respect to the vascular affinity of estrogen as compared to that of the rest of the uterus, to my knowledge there are no data available and this is one of the things that we are speculating about.

I appreciated very much Dr. Herbst's comments because he answered what I would answer in terms of the two-step method versus the newer total nuclear receptor concept. Actually, what we have called cytosol receptors may not actually be in the cytosol, but in terms of interpretation the two-step model seems to be a useful working concept at this point. We must, however, recognize that so-called cytosol receptors indeed may be in the nucleus. We have not done any immunohistologic studies along this line.

Dr. Gant, we have not measured any adrenergic receptors during these studies but I would doubt that they changed significantly since β-adrenergic blockage has minimal effect on the estrogen response.

Histologic and hormonal documentation of the luteinized unruptured follicle syndrome

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Histologic and hormonal documentation of a luteinized unruptured follicle that occurred during a spontaneous menstrual cycle in a rhesus monkey is presented. Frequent (every 2 hours) blood sampling to assess midcycle hormonal dynamics in the monkey with the luteinized unruptured follicle and in five monkeys with an ovulatory stigma revealed significant aberrations in the gonadotropin and steroid hormone profiles associated with a luteinized unruptured follicle. Although the midcycle 17β-estradiol surge was normal, the monkey with the luteinized unruptured follicle demonstrated (1) blunted midcycle bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone surges; (2) absence of disparity in the bioassayable luteinizing hormone:immunoassayable luteinizing hormone ratio during the gonadotropin surge; (3) absence of progesterone and 17α-hydroxyprogesterone secretion during the gonadotropin surge; and (4) delayed and blunted rise in progesterone and 17α-hydroxyprogesterone after the gonadotropin surge. These findings suggest that an impaired luteinizing hormone surge, perhaps mediated by insufficient midcycle progestin secretion, is one possible cause of the luteinized unruptured follicle syndrome. (Am J Obstet Gynecol 1986;154:839-47.)

Key words: Luteinized unruptured follicle, ovulatory stigma, impaired luteinizing hormone surge, midcycle progestin secretion

The concept that the oocyte might become entrapped in a luteinized unruptured follicle was proposed in 1966 to explain the discrepancy in ovulationn and conception rates in patients receiving clomiphene citrate.[1] The current terminology for this condition—the "luteinized unruptured follicle" syndrome—was initially used by Jewelewicz[2] to describe a condition in which "luteinized unruptured follicles secrete progesterone, causing all the progestational changes without actual ovulation." In these women, luteal phase plasma progesterone levels, basal body temperatures, and endometrial biopsy indicated that ovulation had occurred.

In 1978, Koninckx et al.[3] and Marik and Hulka[4] independently presented the first indirect evidence for the existence of the luteinized unruptured follicle syndrome. These authors reported that the luteinized unruptured follicle syndrome could be demonstrated by laparoscopic examination of the ovaries and failure to identify an ovulatory stigma in women during the luteal phase of apparently "ovulatory" cycles. Laparoscopic evidence of the luteinized unruptured follicle syndrome has been supported by failure to demonstrate increased peritoneal fluid volumes and concentrations of progesterone and 17β-estradiol that normally occur in women after follicular rupture and release of the steroid-rich follicular fluid into the peritoneal cavity.[5, 6] Indirect evidence for the luteinized unruptured follicle syndrome has also been suggested on the basis of serial pelvic ultrasound examinations.[7, 8] In these studies, criteria for a luteinized unruptured follicle included loss of a clearly demarcated follicle wall and presence of intrafollicular echoes in the absence of a rapid decrease in follicular size or appearance of free cul-de-sac fluid.

Laparoscopic and ultrasonic evidence for the luteinized unruptured follicle syndrome has not been confirmed by histologic study. Although ovarian biopsy of a putative luteinized unruptured follicle has demonstrated granulosa cell luteinization in women receiving clomiphene citrate, histologic identification of the entrapped ovum was not reported.[7, 9] To our knowledge, histologic demonstration of an oocyte entrapped in a luteinized unruptured follicle has been confirmed experimentally in the rat, rabbit, and monkey only after administration of indomethacin or prostaglandin F antiserum.[10]

Here, we report histologic and hormonal documentation of the luteinized unruptured follicle syndrome

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Fig. 1. Photomicrograph of the luteinized follicle containing an oocyte *(arrow)* with intact germinal vesicle and nucleolus. (Hematoxylin and eosin. Original magnification ×15.)

occurring during a spontaneous menstrual cycle in a rhesus monkey. The unsuspected occurrence of this phenomenon during frequent peripheral blood assessment of midcycle hormone dynamics has proffered one possible cause of this heretofore poorly documented syndrome.

Material and methods

Sexually mature rhesus monkeys (*Macacca mulatta*, n = 6) weighing 4.1 to 6.5 kg and having at least two consecutive menstrual cycles of normal duration (25 to 30 days) were chosen for study. Housing, feeding, and general husbandry practices have been described previously.[11]

Blood was drawn daily beginning on day 8 of the menstrual cycle and the sera were assayed for 17β-estradiol by radioimmunoassay. When serum 17β-estradiol concentrations exceeded 100 pg/ml on two consecutive days, laparoscopy was performed with the animals under light ketamine anesthesia (Vetalar, Parke Davis, Morris Plains, New Jersey; 15 mg/kg intramuscularly) to document the presence of a putative dominant follicle. Monkeys were then fitted with a vest and mobile tether assembly, which permitted placement of a chronic indwelling femoral vein cannula and blood collection without anesthesia. Details of this procedure have been described previously.[11]

After the monkey had recovered from operation, 3.5 ml of blood was collected in sterile heparinized syringes every 2 hours. Plasma was harvested and frozen at −4° C; red blood cells were suspended in 0.9% saline solution and returned to the monkey. Laparoscopy was again performed, with the animals under ketamine anesthesia, 72 to 96 hours after the 17β-estradiol peak to document the presence of an ovulatory stigma. Blood sampling was discontinued on identification of the stigma. One monkey failed to demonstrate a stigma 96 hours after the 17β-estradiol peak. In this animal, laparotomy was performed and the ovary containing the putative dominant follicle was resected and fixed in Bouin's solution. The follicle was serially sectioned every 50 μm, stained with hematoxylin and eosin, and examined microscopically.

Plasma concentrations of luteinizing hormone, follicle-stimulating hormone, 17β-estradiol, progesterone, and 17α-hydroxyprogesterone were measured by radioimmunoassay.[13] Additionally, plasma luteinizing hormone was measured by the rat interstitial cell testosterone in vitro bioassay.[11] The bioassay dose-response curve was linear over the range from 2 to 50 μg. Bioassayable and immunoassayable luteinizing hormone were determined with human pituitary gonadotropin LER 1909-2 used as the standard.

The bioassayable luteinizing hormone surge initiation served as time zero for the temporal analysis of changes in circulating hormones. The bioassayable luteinizing hormone surge initiation and the surge initiation of immunoassayable luteinizing hormone and follicle-stimulating hormone were defined as the first plasma concentrations exceeding the means ± 2 SD of the preceding three values for each gonadotropin. The duration of rise for the gonadotropins was defined as the time interval between the surge initiation and the peak concentration of the respective gonadotropin. The surge duration was the interval from the surge initiation to the time when the postpeak concentration decreased to a value below that of the surge initiation.

Comparisons of time intervals were done with Friedman's nonparametric analysis of variance, since waiting times are often not normally distributed. Doubling times or half-times were computed for each time interval of interest, each hormone, and each monkey using 1n (2)/slope, where the slope was computed from a logarithmic linear regression. Since this ratio is not theoretically expected to be normally distributed, we analyzed the doubling or half-time with the use of Friedman's (to compare the hormones) and Wilcoxon's signed rank (to test if doubling times or half-times were significantly different from zero) nonparametric procedures. In monkeys with an ovulatory stigma, data were summarized by computing the mean and standard error for the five monkeys. The above parameters in

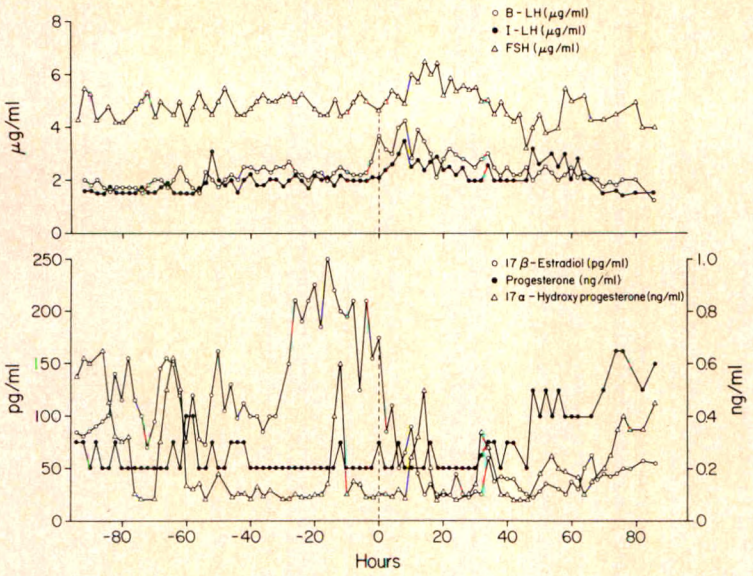


Fig. 2. Composite pattern of plasma gonadotropin *(upper panel)* and steroid *(lower panel)* concentrations measured every 2 hours during midcycle in the monkey with a luteinized unruptured follicle. Values are normalized to the onset of the bioassayable luteinizing hormone surge.

ovulatory monkeys and the monkey with the luteinized unruptured follicle were compared with the use of two-tailed *t* tests.

Results

Histologic examination of the resected ovary clearly demonstrated the presence of an oocyte entrapped in an early corpus luteum (Fig. 1). The oocyte measured 150 μm in diameter and contained an intact germinal vesicle and nucleolus. The cumulus mass was completed dissociated from the oocyte and detached cumulus or granulosa cells were present within the follicle among a fine fibrin network, occasional leukocytes, and erythrocytes.

The granulosa cells in the follicle wall were loosely radially arranged. Occasional areas gave the appearance of streaming into the central cavity. Capillary penetration into the granulosa cell layer was evident. The majority of granulosa cells were spindle-shaped with small nuclei, but some demonstrated an increased cytoplasmic volume and vacuolization. Luteinization of the theca cells was more advanced and a clear demarcation of theca and granulosa cells was present. Rare mitoses were present in both cell layers. Perifollicular stromal luteinization was also noted.

The temporal changes in plasma gonadotropin and steroid concentrations in the monkey with the luteinized unruptured follicle are presented in Fig. 2. The bioassayable luteinizing hormone surge initiation occurred 6 hours before the immunoassayable luteinizing hormone surge initiation and 10 hours before the follicle-stimulating hormone surge initiation. The duration of rise for bioassayable luteinizing hormone (8 hours) was similar to that for immunoassayable luteinizing hormone (6 hours) and follicle-stimulating hormone (8 hours). During this interval, the doubling times for bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone were 12.3, 12.2, and 14.1 hours, respectively. The surge durations for bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone were 20, 12, and 20 hours, respectively. Peak levels of 4.3, 3.5, and 6.5 μg/ml were achieved for bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone, respectively. No significant disparity in the ratio of bioassayable to immunoassayable luteinizing hormone was noted during the gonadotropin surge.

Serum 17β-estradiol concentration in this monkey demonstrated a progressive increase from the onset of blood collection to time zero + 16 hours with a doubling time of 36.6 hours. No significant increase in progesterone occurred before or during the gonadotropin surge; however, progesterone concentrations did gradually increase from time zero + 48 hours to time zero + 86 hours with a doubling time of 41.5 hours. Plasma 17α-hydroxyprogesterone concentrations demonstrated random increases throughout the sampling period but never exceeded 0.6 ng/ml. No sustained elevation in 17α-hydroxyprogesterone concentrations occurred until time zero + 64 hours, at which time 17α-hydroxyprogesterone concentration demonstrated a gradual increase with a doubling time of 17.5 hours.

The dynamic changes in plasma gonadotropins and

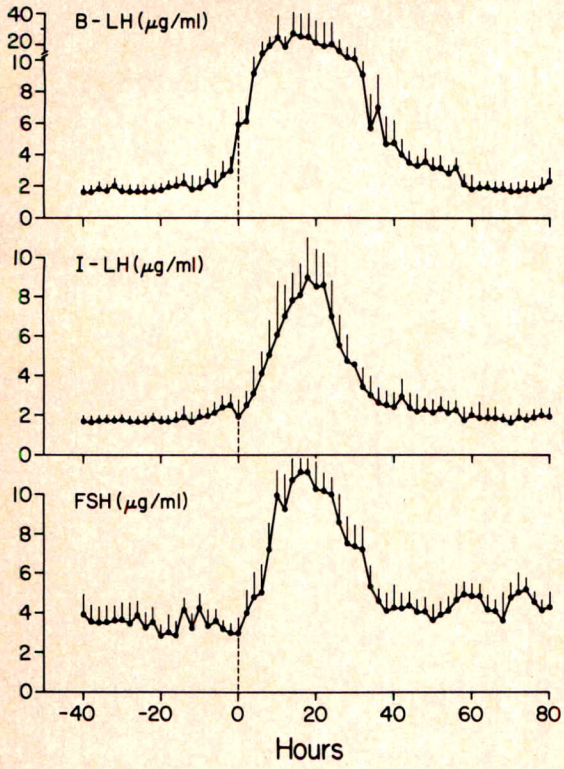


Fig. 3. Composite pattern of plasma gonadotropin concentrations measured every 2 hours during midcycle in the five monkeys with an ovulatory stigma. Values are normalized to the onset of the bioassayable luteinizing hormone surge and expressed as mean ± SEM *(vertical bars)*. (Reprinted from Schenken RS, Werlin LB, Williams RF, et al. J Clin Endocrinol Metab 1985;60:886.)

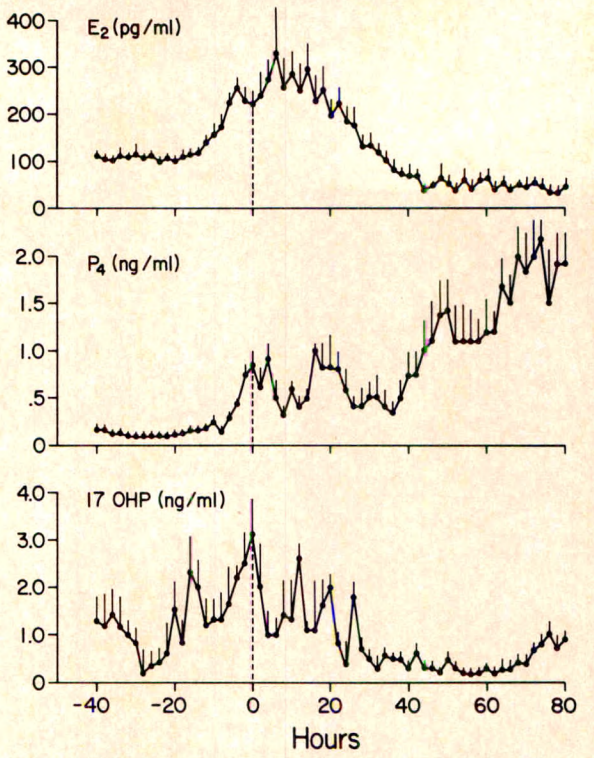


Fig. 4. Composite pattern of plasma steroid concentrations measured every 2 hours during midcycle in the five monkeys with an ovulatory stigma. Values are normalized to the onset of the bioassayable luteinizing hormone surge and expressed as mean ± SEM *(vertical bars)*. (Reprinted from Schenken RS, Werlin LB, Williams RF, et al. J Clin Endocrinol Metab 1985;60:886.)

Table I. Midcycle rise and surge durations of gonadotropins during ovulatory cycles

	Bioassayable luteinizing hormone	*Immunoassayable luteinizing hormone*	*Follicle-stimulating hormone*
Rise duration*	14.8 ± 1.6	9.2 ± 1.6	7.8 ± 1.7
Surge duration*	36.4 ± 4.8†	28.0 ± 2.4	32.8 ± 2.7

*Expressed in hours (mean ± SEM).

†$p < 0.03$ versus values in luteinized unruptured follicle cycle.

steroids in the five monkeys with an ovulatory stigma are presented in Figs. 3 and 4, respectively. In normally ovulatory monkeys, the immunoassayable luteinizing hormone and follicle-stimulating hormone surge initiations occurred 5.6 ± 1.2 hours (±SEM) and 7.0 ± 1.5 hours (±SEM), respectively, after the bioassayable luteinizing hormone surge initiation. During this interval, the mean doubling times (±SEM) for the bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone were 6.6 ± 1.3, 7.3 ± 1.2, and 8.0 ± 1.3, respectively. The midcycle rise and surge durations for gonadotropins are presented in Table I. Although the doubling times and temporal relationships among gonadotropins were similar in these monkeys and the one with a luteinized unruptured follicle, the surge duration of bioassayable luteinizing hormone and the secretion (area under the curve) of bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone in ovulatory monkeys were significantly greater than those observed in association with the luteinized unruptured follicle. In contrast to the monkey with the luteinized unruptured follicle, the bioassayable luteinizing hormone : immunoassayable luteinizing hormone ratio in monkeys with an ovulatory stigma demonstrated a significant increase from near unity before the bioassayable luteinizing hormone surge initiation to approximately 6 : 1 at the acme of gonadotropin secretion.

The doubling time of plasma 17β-estradiol, peak 17β-estradiol concentrations, and 17β-estradiol secretion (area under the curve) in ovulatory monkeys were similar to those observed in the monkey with the luteinized unruptured follicle. However, progesterone and 17α-hydroxyprogesterone concentrations increased >0.6 and 2.0 ng/ml, respectively, before or dur-

ing the bioassayable luteinizing hormone surge only in monkeys with an ovulatory stigma. This initial increase in progestins in ovulatory monkeys was followed by a plateau phase that lasted 24 to 48 hours; thereafter, sustained increases in progesterone and 17α-hydroxyprogesterone were initiated within 26 and 68 hours, respectively, of the bioassayable luteinizing hormone surge initiation. Before discontinuation of blood collections, progesterone and 17α-hydroxyprogesterone concentrations were in excess of 1.5 and 0.8 ng/ml, respectively, in all monkeys with an ovulatory stigma.

Comment

Numerous studies have recently drawn attention to the occurrence of the luteinized unruptured follicle syndrome in women with endometriosis or unexplained infertility and after induction of ovulation with clomiphene citrate.[3, 7, 12] In these studies, failure of ovum release was documented either by absence of an ovulatory stigma at laparoscopic visualization of the ovary or by failure to identify characteristic ultrasonic features of ovulation. Although ovum retention has been demonstrated in humans with ruptured follicles and in laboratory animals receiving antiprostaglandin treatments, our report constitutes the first histologic and hormonal documentation of the luteinized unruptured follicle syndrome during a spontaneous menstrual cycle.[10, 13]

Histologic examination of the putative luteinized unruptured follicle demonstrated characteristic features of an early corpus luteum without an ovulatory stigma. The observation of capillaries penetrating the granulosa cell layer is, according to the criteria of Corner,[14] consistent with a corpus luteum 2 days after ovulation. Clearly, demonstration of an entrapped oocyte with an intact germinal vesicle and nucleolus is evidence that "ovulation" did not occur.

Frequent blood sampling and assessment of midcycle hormonal changes in the monkey with the luteinized unruptured follicle and in monkeys with an ovulatory stigma revealed significant aberrations in the gonadotropin and steroid hormone profiles associated with the luteinized unruptured follicle syndrome. Although the midcycle 17β-estradiol surge and temporal relationship between gonadotropins were not abnormal, the monkey with the luteinized unruptured follicle did demonstrate several abnormalities, when compared with normal ovulatory monkeys, which included: (1) blunted midcycle bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormone surges; (2) absence of disparity in the bioassayable luteinizing hormone:immunoassayable luteinizing hormone ratio during the gonadotropin surge; (3) absence of progesterone or 17α-hydroxyprogesterone increases in conjunction with the gonadotropin surge; and (4) delayed and blunted rise in progesterone and 17α-hydroxyprogesterone after the gonadotropin surge. Although previous studies have suggested that midcycle hormonal patterns are normal in women with a luteinized unruptured follicle diagnosed by laparoscopy, our findings suggest significant abnormalities in midcycle gonadotropin and steroid dynamics in association with histologic confirmation of a luteinized unruptured follicle.

The necessity for a timely midcycle luteinizing hormone surge to trigger the complex process of ovulation is accepted. However, the precise quantitative and qualitative changes in midcycle luteinizing hormone secretion required to initiate ovulation remain unknown. The midcycle gonadotropin surge, particularly the bioassayable luteinizing hormone component, observed in association with a luteinized unruptured follicle was significantly blunted as compared with that in ovulatory monkeys. The cause of this impaired luteinizing hormone surge was not related to inadequate follicular 17β-estradiol secretion but was possibly due to insufficient progestin secretion. Recently, studies have demonstrated that progesterone will hasten the onset of the immunoassayable luteinizing hormone surge and that progesterone is required to establish the normal dimensions of an 17β-estradiol–induced gonadotropin surge.[15, 16] Since no obvious increase in progesterone or 17α-hydroxyprogesterone occurred either before or during the gonadotropin surge in the monkey with a luteinized unruptured follicle, insufficient progestin secretion may have mediated the blunted luteinizing hormone surge and subsequent unruptured follicle. The reason for insufficient progestin secretion at midcycle is unclear.

Midcycle progesterone secretion has also been implicated as a significant factor in fostering rupture of mature follicles. Although progesterone has been shown to induce ovulation in rat, rabbit, and hamster ovaries under various experimental conditions, there is no evidence to suggest that progesterone in the absence of gonadotropins can induce ovulation in vivo or in vitro.[17] Consequently, insufficient midcycle progesterone as a direct mediator of follicular rupture cannot be implicated as the sole cause of failure of oocyte release.

Others have postulated that the luteinized unruptured follicle syndrome is caused by transient psychological stress and attendant hyperprolactinemia that induce premature luteinization of the follicle in infertile women.[12] Another proposed mechanism suggests that follicular prostaglandin $F_{2\alpha}$ deficiency may result in failure of follicular rupture. Unfortunately, we cannot address these hypotheses because serum prolactin and follicular prostaglandin concentrations were not determined in our study.

Several distinct events make up the ovulation process, including ovum maturation and resumption of meiotic division, follicular luteinization and characteristic steroidogenic activity, and the complex process of follicular rupture and oocyte release. Although these events are likely controlled by independent mechanisms, the trigger for each event appears to be the luteinizing hormone surge. Our observation of impaired bioassayable luteinizing hormone and immunoassayable luteinizing hormone surges, possibly mediated by insufficient midcycle progestin secretion, in asssociation with an oocyte arrested in the first meiotic division and entrapped in a luteinized follicle, suggests that the luteinizing hormone trigger was sufficient to induce follicular luteinization, yet inadequate to permit resumption of meiotic division, follicular rupture, and release of a mature oocyte. These findings should be interpreted with caution since only one case of luteinized unruptured follicle has been unequivocally documented and because numerous other factors (for instance oocyte maturation inhibitor, nonsteroidal luteinization inhibitors and stimulators, nonsteroidal gonadotropin regulatory substances, prostaglandins, proteolytic enzymes, and cyclic adenosine monophosphate) may have a significant, yet unappreciated, role in the etiology of the luteinized unruptured follicle syndrome. Further, the abnormal midcycle hormonal patterns observed in this study may represent only one of several possible mechanisms of the luteinized unruptured follicle syndrome.

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Editor's note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Nathan Kase, New York, New York. Perhaps the most important concept in the understanding of ovarian physiology that has emerged during the past decade is the realization that the preovulatory ovarian follicle directly controls much of its physiologic fate, that is, achieving ovulation and corpus luteum function. By steroidal and nonsteroidal extragonadal messages the follicle directs and abets anterior pituitary gonadotrope cells to synthesize and release timely and appropriate concentrations of the gonadotropins follicle-stimulating hormone and luteinizing hormone. Similarly, the follicle exerts inhibitory dominance over maturation of other competing follicles in both the ipsolateral as well as the contralateral ovary by as yet uncharacterized suppressive hormonal messages. Finally, the endocrine microenvironment of the dominant preovulatory follicle induces and synchronizes the crucial events of ovulation—resumption of oocyte meiosis, the proteolytic disruption of follicle structural integrity leading to ovum extrusion, and the reorganization and vascularization of the granulosa cells preparatory to significant progesterone secretion.

Progesterone appears to play a signficant role in several of these midcycle events. Progesterone is influential in augmenting the gonadotropic reaction to gonadotropin-releasing hormone and secretion of both follicle-stimulating hormone and luteinizing hormone.

The possible role of intragonadal progesterone in synchronizing meiosis with ovum extrusion is intrigu-

ing. Although not established for mammalians, studies suggest two new biologic functions for progesterone: First, progesterone acts on the oocyte membrane to induce formation of a cytoplasmic protein messenger, which migrates to the oocyte nucleus and causes resumption of meiosis I. Second, progesterone, again operating via the oocyte membrane, appears to promote the appearance of another protein, which reduces or reverses the action of the granulosa cell oocyte meiosis inhibitor, which has arrested meiosis since the differentiation of the gonad in utero. In this way, during the luteinizing hormone surge, progesterone production in the microenvironment of the follicle causes resumption of meiosis at the same time that secretion of progesterone augments gonadotropin secretion. Extrapolation of this process to the human is premature. However, in vitro fertilization–embryo transfer studies such as those of Seibel et al. (Am J Obstet Gynecol 1982;142:568), correlated the luteinizing hormone surge with the progress of first meiotic division. This work demonstrated a relationship between the initiation of the luteinizing hormone surge with "germinal vesicle" condensation and the completion of meiosis I following the peak of luteinizing hormone concentrations.

In the paper "Histologic and hormonal documentation of the luteinized unruptured follicle syndrome," Schenken et al. have offered additional information on the importance of progesterone in the mechanics of ovulation in the rhesus monkey. This study provides histologic and hormonal documentation of a luteinized unruptured follicle occurring in a spontaneous menstrual cycle in the primate. By frequent blood sampling the midcycle hormonal dynamics in the animal with luteinized unruptured follicle are compared with similar measurements in five monkeys assumed to have ovulated normally. Significant midcycle observations include: normal estradiol; blunted concentrations of bioassayable luteinizing hormone, immunoassayable luteinizing hormone, and follicle-stimulating hormones; reduced bioassayable luteinizing hormone:immunoassayable luteinizing hormone ratios in the luteinized unruptured follicle syndrome; absence of progesterone and 17α-hydroxyprogesterone secretion during the gonadotropin surge, and a delayed as well as blunted rise in these steroids after the gonadotropin surge. The data suggest that an impaired bioassayable luteinizing hormone surge, possibly mediated by insufficient progestin secretion by the preovulatory follicle, might be a component in the etiology of the luteinized unruptured follicle syndrome. Although limited to its focus, this is extremely important work that demands confirmation and extension to other rhesus cycles and to the woman.

Certain limitations in the data must be noted and discussed before premature assumptions are made. Among the uncertainties that need clarification are the following:

1. Although these investigators are among the leaders in primate reproduction research, can they assure us that external visualization by laparoscopy is sufficient to document the presence of a dominant follicle in an ovary?

2. Are there data to validate the assumption that the presence of a stigma in the "normal" ovary is in fact proof of ovum extrusion?

3. Through their ingenious work with luteectomy these workers have shown the importance of the dominant follicle in the subsequent timing of the resumption of follicle maturation and next ovulation. We are not given information sufficient to show that oophorectomy in the animal with a luteinized unruptured follicle was associated with the expected delay of resumption of the next cycle. Hormonal data covering the remainder of the cycle in this animal are necessary to rule out the presence, albeit delayed, or an unnoted dominant follicle in the contralateral ovary but are not available in the manuscript.

4. Although sample frequency appears to be adequate (every 2 hours), it has recently been suggested that more frequent analyses might have displayed more detailed information with respect to the "appropriateness" of progesterone and gonadotropin secretions.

5. The investigators have shown the hormonal and structural details of a cycle in which an impaired dominant follicle remained unruptured and attenuated in what appeared to be a "normal cycle in a normal animal." To suggest that this offers documentation of a luteinized unruptured follicle syndrome is beyond the scope of the data. Was this animal infertile? Had there been previous cycle irregularities in the animal? In short, did this animal share the infertility of "undiagnosed etiology" we require in the human luteinized unruptured follicle syndrome?

6. The authors suggest that a midcycle hormonal abnormality was the proximal cause of the unruptured follicle in this animal. However, based on the histologic characteristics of the follicle represented in the authors' Fig. 1, there do not appear to be sufficient numbers of healthy granulosa cells to support the contention that this was an otherwise normal "unruptured preovulatory follicle." Are we seeing midcycle changes that are secondary to earlier menstrual or postmenstrual aberrations leading to failure of adequate preparation of granulosa cell maturation and function? Indeed, these workers have provided precisely this insight as the basis for the late consequences of early cycle application of nonsteroidal follicle maturation inhibitors.

7. We are asked to relate midcycle hormonal events to midcycle follicle histologic characteristics as seen only in the affected follicle. Would it have been useful to compare histologic characteristics of the affected follicle with those of follicles in normal "cases"?

8. Regarding the study design, the authors chose to preserve the affected follicle intact to show the entrapped ovum in a very convincing manner. They succeeded but at the expense of the lost opportunity to scrutinize the biochemical mechanisms at the intrafollicular antral level, thereby correlating circulating hormones with their microenvironment concentrations. Were the priorities inherent in the study design improperly "tilted" to display rather than interpret events?

In conclusion, and with the aforementioned quibbling put in proper perspective, this is an important insight into ovarian pathophysiology. The caution to restrain from extrapolating these interesting data to clinical reality is worth reemphasizing. However, without this kind of pathfinding, horizon-expanding work, typical of this investigative team, we would face the dismal frustrating prospect of wrestling with problems affecting the aspirations of our patients with directionless ignorance.

DR. E. STEWART TAYLOR, Denver, Colorado. I feel a little embarrassed to talk about something I know nothing about, but I found this a very interesting article, particularly for the physician who sees infertility patients in his office where everything appears to be normal but no pregnancy occurs. I think this does give thoughtful information about the inadequate luteal phase. It also sheds some light on why the empiric use of Clomid in a patient who appears to be normal is sometimes a successful event.

DR. M. **YUSOFF DAWOOD,** Chicago, Illinois. Could you tell us the length of the luteal phase of the monkey with the unruptured follicle?

DR. FRANK C. GREISS, JR., Winston-Salem, North Carolona. I am an ignorant, or maybe more so, as Dr. Taylor claimed to be, of this area. However, I wonder why more attention hasn't been focused on the early appearance of the estradiol surge and the fact that no immediate responses followed this. Could you explain this observation.?

DR. SCHENKEN (Closing). I greatly appreciate the pertinent commentary and probing questions. I agonized over many of these same questions when we were putting this manuscript together. I will try to address the questions in the order they were asked. First, is laparoscopic visualization of the ovary sufficient to identify the presence of a dominant follicle? We feel that laparoscopic visualization of the ovary is adequate when it is performed within 4 to 5 days of the time of ovulation. Before this time it is difficult to identify the dominant follicle. However, one can rely on measuring ovarian vein estrogen secretion to identify the ovary containing the dominant follicle.

Are there data to validate the assumption that the presence of a stigma is proof of ovum extrusion?

I am unaware of any studies that have looked at this question systematically. In women undergoing in vitro fertilization, oocytes have been aspirated from follicles bearing obvious stigmas. However, I think it is the experience of most in vitro fertilization programs that oocytes are rarely recovered from either follicles bearing a stigma or corpora hemorrhagica.

An appropriate pun here might be added: "A stigma will do to beat a dogma."

Dr. Kase also expressed concern that hormone data for the remainder of the cycle were not obtained and thus an unnoted dominant follicle in the contralateral ovary may have been present. Because of the rigorous blood sampling and multiple operations performed in this study, we felt that continued studying of this animal would place the monkey at unnecessary risk. The monkey with the unruptured follicle did have menses approximately 31 days after the oophorectomy. In view of previous data demonstrating that recruitment and ovulation of the dominant follicle require approximately 14 days, it is most likely that a new dominant follicle was recruited after the termination of our study.

In regard to the question on the monkey's menstrual cycle and fertility history, the monkey with the unruptured follicle conceived twice in a 2-year period before the study and had normal menstrual cycles for 8 months before our study. Consequently, I do not think this monkey had a diagnosis of unexplained infertility. Further, until we can ascertain whether the luteinized unruptured follicle syndrome occurs repetitively in each cycle, thus causing infertility, or whether the luteinized unruptured follicle is an isolated, infrequent event, I cannot agree with Dr. Kase's suggestion that infertility of unexplained etiology is a prerequisite for diagnosing the luteinized unruptured follicle syndrome.

The suggestion that an early follicular phase aberration led to a failure of normal mid cycle granulosa cell function, that is, progesterone secretion, is cearly possible. Indeed, the early follicular phase administration of porcine follicular fluid, which contains a nonsteroidal gonadotropin-inhibiting factor, will result in an inadequate luteal phase. We have also observed that monkeys with endometriosis and unruptured follicles commonly have a short luteal phase with inadequate progesterone secretion. Thus it is entirely possible that an early follicular phase abnormality may have been related to the genesis of this syndrome.

I completely agree with the recommendation that the histologic features of the unruptured follicle should have been compared with those of follicles demonstrating an ovulatory stigma. Unfortunately, as luck would have it, the monkey with the unruptured follicle was not identified until 4 days after all the other monkeys had completed the study. Consequently, to go back and resect the follicle would not have been temporally significant.

Dr. Kase also appropriately pointed out that our decision to histologically document the presence of an oocyte entrapped in a luteinized follicle was made at the expense of evaluating the "biochemical mechanisms" at the intrafollicular antral level. Accordingly, his question was, were the priorities in the study improperly tilted to display rather than interpret events? Clearly, our study was designed to document an event, but I do not think this was improper. In view of the fact that an oocyte entrapped in a luteinized unruptured follicle has never been documented histologically, it seems evident to me that a study designed to document unequivocally the existence of a syndrome should take precedence over a study designed to evaluate events associated with an undocumented syndnrome.

With regard to Dr. Greiss' question, the estrogen

surge in the monkey with the unruptured follicle did appear to occur earlier in relation to the bioassayable luteinizing hormone surge as compared with that in monkeys demonstrating an ovulatory stigma. However, with all the statistical machinations we used, we could not say that this was a significantly earlier occurrence of the estradiol surge.

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An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries

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One hundred twenty-five consecutive pregnancies conceived in vitro resulted in 100 deliveries of 115 babies. There were 23 clinical abortions (18.4%) and two tubal pregnancies. During the same interval 30 preclinical pregnancies occurred, but these pregnancies did not progress. There were 26 multiple pregnancies (37.1%) before the twelfth week; these reduced spontaneously to 14 (22.2%) multiple births at delivery. Eight infants were delivered prematurely, and three of these died. Three babies had some congenital abnormality. Vaginal bleeding occurred during pregnancy in 59% of patients. Cesarean section was the method of delivery in 56% of patients. Other complications of pregnancy were similar to those of comparable populations. (Am J Obstet Gynecol 1986;154:848-54.)

Key words: In vitro fertilization, pregnancy outcome, multiple pregnancy, infertility

More than 1500 deliveries of babies conceived in vitro have occurred throughout the world. There is yet no reported detailed analysis of the obstetric experience of all of a substantial number of pregnancies conceived in a single clinic. Steptoe[1] reported pregnancy outcomes in 242 deliveries.

Material and methods

Between April 15, 1981, and August 25, 1984, 125 clinical pregnancies were conceived in vitro at the Howard and Georgeanna Jones Institute for Reproductive Medicine. The methods of stimulation and monitoring of the patients under stimulation have been previously reported.[2-4] All but two received gonadotropins in supraphysiologic concentrations in an attempt to obtain multiple preovulatory oocytes for fertilization. Sixty-eight of these patients were stimulated with human menopausal gonadotropin (hMG). In 45 patients a combination of hMG and follicle-stimulating hormone (FSH) supplementation was used. In 12 patients FSH alone was used.

Of the 100 deliveries resulting, 12 were delivered at the Eastern Virginia Medical Center. Each of the other pregnancies, delivered throughout the United States and in three foreign countries, was evaluated through a questionnaire and personal communication with the patient and/or physician as indicated to obtain as complete information as possible. All babies in this series are being brought back for multidiscipline diagnostic evaluation for congenital abnormalities and for neurodevelopmental assessment in the Pediatric Department of the Eastern Virginia Medical School after 1 year of age or as soon thereafter as practical. Twenty-one babies have so far been evaluated in this manner.

During this same interval 30 additional patients achieved preclinical pregnancies. These are diagnosed (1) when the quantitative human chorionic gonadotropin (hCG) serum concentration rises to ≥10 mIU/ml on luteal days 11 to 13, (2) when a second hCG value is further elevated, (3) when these are accompanied by a second rise of serum estradiol and/or progesterone, (4) when the hCG level drops again to ≤5 mIU/ml or less before 14 days after the expected menstrual period, and (5) when menses is delayed no more than 14 days. No products of conception are encountered and no curettage is performed.

Table I. Outcome of 125 pregnancies conceived in vitro, April 15, 1981, to August 25, 1984*

Deliveries		100
Babies	115	
Clinical abortions < 20 wk		23
Tubal pregnancies		2
Total		125

*During this interval 30 preclinical pregnancies occurred but did not progress.

Results

The 125 pregnancies resulted in 100 deliveries, which produced 115 babies (Table I). There were 23

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Table II. Clinical abortions among 125 pregnancies conceived in vitro according to method of ovulation stimulation

	hMG	*FSH + hMG*	*FSH*	*Total*
Deliveries	60	32	8	100
Clinical abortions	8(11.7%)	11(25%)	4(33%)	23(18.4%)
Ectopic pregnancies	0	2	0	2
Total	68	45	12	125

Table III. Total loss of pregnancy among patients with serologic evidence of trophoblastic implantation, proliferation, and function (N = 155), April 15, 1981, to August 25, 1984

	Stimulation			
	hMG	*hMG + FSH*	*FSH*	*Total*
Total pregnancies	88	51	14	155
Clinical abortion	8(9%)	11(21%)	4(29%)	23(15%)
Preclinical abortion	20(23%)	6(12%)	2(14%)	28(18%)
Pregnancy loss	28(31%)	17*(33%)	6(43%)	51(33%)

Values are expressed as a percentage of total endocrinologically detected pregnancies.
*Plus two tubal pregnancies.

Table IV. Method of delivery in 100 deliveries of pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

Method of delivery	*n*	*%*
Cesarean section	56	56
Vaginal	42	42
Vaginal + cesarean section	2	2

Table V. Indications for cesarean section in 56 cases

Indication	*n*
Cephalopelvic disproportion + failure to progress	17
Multiple pregnancy	10
Large baby + cephalopelvic disproportion	7
Breech presentation	5
Repeat cesarean section	2
Preeclampsia + premature rupture of membranes with failed induction	3
History of bilateral cornual reimplantation	1
Total placenta previa	1
Low-lying placenta	1
Intrapartum fetal distress	2
Intrauterine growth retardation	1
Failure to remove cerclage suture applied at 7 wk	1
Indications not stated or not clear	5
Total	56

clinical abortions before the twentieth week of gestation and two tubal pregnancies.

Abortions. The overall abortion rate in this series was 18.4%. Table II indicates the distribution according to method of stimulation. If preclinical pregnancies conceived during the same interval are considered as a proportion of all pregnancies, the rate of this occurrence is 19% (Table III). With this inclusion the total pregnancy loss is 33%.

Sex of babies. Sixty-four of the 115 babies were male. Fifty-one were female.

Method of delivery. Table IV indicates the method of delivery. In one case a premature breech infant was delivered by cesarean section after vaginal delivery of a premature stillborn infant. In another an abruptio placentae resulted during a manual attempt to rotate from breech to vertex after the vertex delivery of the first twin. The indications given for cesarean section are listed in Table V.

Prematurity. One pregnancy terminated at 25 weeks. An intramural myoma was present. Seven other pregnancies terminated before 36 weeks. Two of these were twin pregnancies, and one a triplet pregnancy. Two other patients among these seven had "T"-shaped uteri characteristic of intrauterine diethylstilbestrol exposure (Table VI).

Fetal losses. There were three deaths in infants delivered prematurely.

A 750 gm male was delivered of a 36-year-old mother at 25 weeks' gestation, 12 hours after spontaneous premature rupture of the membranes. After 5 weeks in neonatal intensive care and a septic course, small bowel resection for enterocolitis was carried out and death occurred while the infant was in septic shock at 6 weeks. No congenital abnormalities were found. The patient had an intramural uterine myoma, which was 1.5 cm

Table VI. Outcome of 100 deliveries of pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

Length of gestation (wk)	*No. of pregnancies delivered*	*No. of living babies*
20-28	1*	0
28-36	7†	8
36-42	91	103
42	1	1
Total	100	112

*A 740 gm male infant died at 6 weeks.

†A 1360 gm stillborn first twin (second twin survived) and a 1300 gm stillborn infant at 29 weeks (intrauterine death).

Table VII. Multiple pregnancies* among 100 deliveries of pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

	< 12 wk	*At delivery*
Twins	23	13
Triplets	3	1
Total	26(37.1%)	14(20.2%)

All multiple pregnancy deliveries occurred among the multiple transfers.

Another triple sac was reduced to a singleton and subsequently aborted. One single conceptus produced two sacs but was reduced to a singleton at delivery.

*Two or more conceptuses implanted (N = 69).

in diameter before pregnancy and enlarged considerably during pregnancy.

A 1620 gm male infant, the first twin, was stillborn at 29 weeks' gestation (mother age 34). Autopsy revealed no structural abnormalities. The second twin (1410 gm) was delivered by cesarean section and survived. There had been vaginal bleeding for the last 3 months of pregnancy. Five four-cell conceptuses and one two-cell conceptus had been implanted. There was marginal placenta previa.

A 1300 gm male stillborn infant was delivered at 29 weeks' gestation. Blood pressure had been elevated to levels of 200/110 mm Hg during the previous week. Weight gain had been 35 pounds. Prenatal visits were infrequent. Fetal movements had ceased and the fetal heart could no longer be detected 2 days before delivery. Autopsy revealed no congenital abnormalities.

Ectopic pregnancies. Ectopic pregnancy necessitating salpingectomy occurred in two patients.

Multiple pregnancies. There were 26 multiple pregnancies (26%) as determined by ultrasound before 12 weeks. Sixty-nine patients had had two or more conceptuses transferred. The average number of conceptuses transferred overall, including immature fertilized eggs, was 3.2. One was monozygotic, since only one conceptus had been implanted. Nine twin sacs were reduced to one sac. One triple sac was reduced to twins. Another triple sac was reduced to a single pregnancy at delivery. One was reduced to a single abortion. The resulting multiple delivery rate was 14% (Table VII). Ten of these 12 reductions before delivery were accompanied by vaginal bleeding. In addition, three of the 23 abortions were recognized to be twin pregnancies. One of these twin sacs that aborted occurred in another pregnancy in the same patient, who previously had reduction of twin sacs to a single pregnancy and who again had only a single conceptus transferred.

Table VIII. Vaginal bleeding during 91 of 100 pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

Trimester bleeding began	*n*
First	49
Second	3
Third	2
Total bleeding	54 (59%)
No bleeding	37
Total	91

Table IX. Location of placenta by ultrasound in 71 of 100 pregnancies, April 15, 1981, to August 25, 1984

Location	*n*	*%*
Anterior	31	43
Posterior	19	27
Fundal	18	26
Complete placenta previa	1	1
Low-lying placenta	2	3

Vaginal bleeding. Fifty-nine percent of the 91 patients from whom an adequate history was obtained had vaginal bleeding during pregnancy. One had a placenta previa and another marginal placenta previa (Table VIII). Vaginal bleeding was as common among patients with single transfers as among those with multiple transfers. Bleeding frequently occurred during a period of days or weeks. One patient who was delivered at term was advised to have a curettage performed in the third month because of the quantity of persistent bleeding.

Placental location. The location of the placentas in the 71 patients from whom this information could be recovered is listed in Table IX.

Congenital abnormalities. Congenital abnormalities occurred in three infants.

A 3530 gm male infant born to a 41-year-old mother with no family history of neural tube defect had a 6 to 7 cm myelomeningocele of the lumbar spine. At the age of 6 months a nonemergency ventricular shunt was carried out because of evidence of incomplete drainage.

Table X. Amniocentesis among 100 pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

Patients ≥ age 35	39
Amniocentesis	27
No amniocentesis	12
Anterior placenta (age 39)	1
Aged 35 and 36	2
Elected no amniocentesis	4
Reason unknown	5

Table XI. Pregnancy-induced hypertension among 86 of 100 pregnancies conceived in vitro, April 15, 1981, to August 25, 1984

Severe PIH at 29 wk, delivered by CS	1
Severe PIH at 29 wk, fetal death in utero	1
Moderate PIH, magnesium sulfate given	3
Mild PIH	5
Total	10(12%)

PIH = Pregnancy-induced hypertension; CS = cesarean section.

Development appears normal. There is a significant neurological deficit in the lower extremities.

A 3960 gm male infant, delivered of a 32-year-old para 0 woman, was found soon after birth to have a small congenital membranous ventricular septal defect. This did not cause any symptoms or require treatment. When this child returned at 1 year of age and was subjected to the intensive evaluation, ultrasonography detected a stricture at the ureteropelvic junction. This was successfully repaired.

A 3500 gm male infant, born to a 37-year-old para 0 woman, was found at birth to have absence of much of the external ear on the right side (microtia). Hearing is normal.

No other abnormalities have been found among the 115 babies delivered, including 21 intensively evaluated babies.

Amniocentesis. Amniocentesis was performed in 27 of 39 patients whose age was ≥35 years. All chromosome analyses were normal (Table X).

Abortuses. Nine abortuses were analyzed for chromosome content. Seven were normal. Two showed mosaicism as follows: (1) 46,XX/47,XX,15+; (2) 46,XX/46,XX,15−,t(15,21)(p11,q11).

Other complications of pregnancy. Ten of the 86 patients from whom a specific history could be obtained concerning pregnancy-induced hypertension had experienced this complication. One fetal death was associated with severe pregnancy-induced hypertension at 29 weeks (Table XI). Apgar scores are displayed in Table XII.

Weights of babies. The weights of 85 singleton babies are plotted in Fig. 1. With use of the tables designed

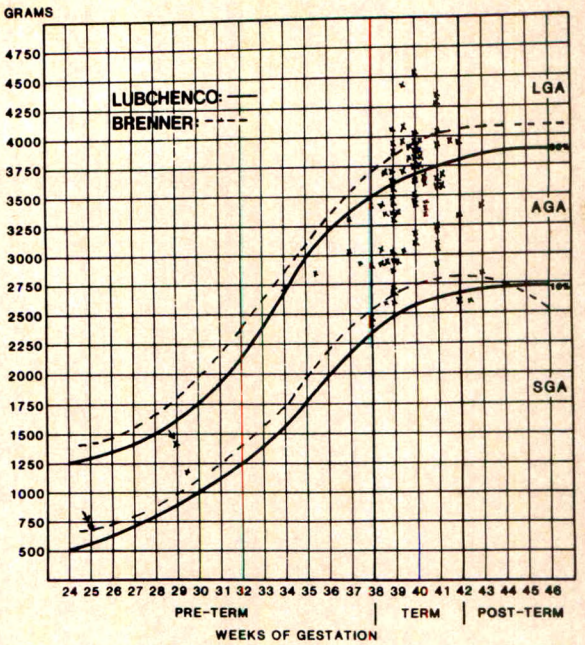


Fig. 1. Fetal growth profile with weights of 85 singleton babies. Appropriate for gestational age *(AGA)*, 65 (77%); small for gestational age *(SGA)*, 6 (7%); large for gestational age *(LGA)*, 14 (16%).

Table XII. Apgar scores among 81 babies conceived in vitro, April 15, 1981, to August 25, 1984

	No. of babies
Stillborn	2
Apgar scores <7 at 1 min	5
Apgar scores <7 at 5 min	0

by Brenner et al.,[6] 14 (16%) qualified as large for gestational age. Six (7%) were small for gestational age.

Comment

In general, the obstetric results in these 100 patients were consonant with expectations when their age and other special characteristics are considered.

No complications uniquely consequent to this method of conception can be identified beyond the 59% incidence of vaginal bleeding and the higher multiple pregnancy rate.

The higher incidence of induced multiple pregnancies inherent in multiple-conceptus transfer predisposes to a higher incidence of prematurity and fetal loss, as well as abnormality. One fetal loss, on the basis of incomplete information, appears to have been a preventable obstetric accident.

The incidence of prematurity, which was responsible for another fetal loss, appears comparable to that of general experience when multiple pregnancy and intrauterine abnormalities are considered.

The examination of all of these babies for congenital abnormalities has been intense and is probably accurate. The three babies having major abnormalities among the 115 babies described here are consistent with results in studies of larger groups reporting incidences from 2.1%[7] to 3.3%,[8] but final conclusions must await larger numbers. The increased proportion of multiple pregnancies will predispose to a higher proportion of major abnormalities among babies conceived in vitro. The intensive multidiscipline evaluation that is being conducted in all infants conceived in vitro in this program will yield more thorough information, but controls may be difficult to locate. Compliance has been near 100%.

The very high cesarean section rate (56%) probably reflects some concern for the "premium" nature of these pregnancies in addition to the increased numbers of multiple pregnancy, older maternal ages, and increased pregnancy-induced hypertension. It is identical with that reported by Steptoe.[1]

The incidence of pregnancy-induced hypertension in this group probably reflects the older ages, the primigravidity, and the nature of this disease. Evidence of casual obstetric care of some of these pregnancies that were conceived with such difficulty suggests a need to reinforce advice to seek quality care.

The inherent inefficiency of human reproduction and particularly the embryonic loss between fertilization and clinical pregnancy has been explored by Leridon,[5] Edmonds et al.,[9] and Miller et al.[10] Edmonds et al. found that 198 exposures in ovulatory normal volunteers produced 118 (59.6%) chemically detectable pregnancies, but there were only 51 (25.6%) clinically detectable pregnancies and 45 (22.5%) term pregnancies.

The proportion of preclinical pregnancies in this in vitro fertilization series is of somewhat less magnitude and represents, in part, less sensitive detection. The clinical abortion rate of 18.4% among the pregnancies here reported is comparable to that occurring naturally under similar scrutiny. Steptoe[1] reported a clinical abortion rate of 32% among 726 conceptus transfers.

The overall rate of achieving a live birth from transfer of one or more conceptuses in the Norfolk program in the year preceding April, 1985, is 18%, or 4% less than the normal experience of Edmonds et al. During that year the pregnancy rate among 136 cycles in consecutive new patients having the preferred protocol stimulation (hCG/hMG) was 32.3% of all conceptuses transferred.

Improvement year by year has been slow but steady. With increasing knowledge concerning stimulation techniques to produce more consistently two or more fertilizable ova of good quality and with better patient selection techniques, it is quite reasonable to expect that the natural experience may be significantly exceeded.

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Discussion

Dr. Charles R. Brinkman III, Los Angeles, California. This is a very interesting report on the outcome of 127 women who achieved clinical pregnancies after in vitro fertilization. As a perinatologist, I will focus my comments on some of the perinatal and demographic aspects of this paper.

Of the women who carried their pregnancies beyond 20 weeks, 39% were older than 34 years of age. While this percentage is quite high, undoubtedly because of the infertility history, it is interesting to look at the trends occurring regarding maternal age and childbirth. In California, between 1978 and 1982, there was a 1.4% increase in births to women over the age of 34, from 5.2% of 357,000 births to 6.6% of 432,000 births.[1] During the same period, there was a 1.5% increase in Los Angeles County. For comparison, I selected two hospitals that serve communities inhabited by a high proportion of career-oriented professionals. In these communities, the percentage of births to women over 34 has increased from 10% to 15.4% in the same 5-year period. This particular group of pregnant women is usually considered to be at increased risk for many of the medical complications encountered during pregnancy and may account for the 12% rate of pregnancy-induced hypertension observed in this report.

The second facet I wish to discuss is that of a 54% primary cesarean section rate; of the women undergoing primary cesarean birth, apparently half had a trial of labor while half apparently did not, resulting

in an elective primary cesarean section rate of 25%. During 1981, for comparison, in California and Los Angeles County, the primary cesarean birth rate was 10.9% and in the two selected hospitals previously mentioned, it was 17.9%. In addition, Cohen et al.[2] recently reported a primary cesarean rate of 13% in 60 infertility patients managed by maternal-fetal medicine subspecialists. While there is no queston that these are "premium" pregnancies, I believe we would all agree that every pregnancy should receive the level of care necessary to maximize successful outcome for mother and infant. I am not sure that this liberal use of cesarean birth is necessary to meet that aim.

The last point I wish to make has to do with the perinatal mortality rate of this small series. The fetal death rate is 17.4 per 1000, which is almost three times the rate in California for 1981. There were no neonatal deaths, as one infant survived 6 weeks and should be classified as an infant death. Therefore, the perinatal mortality rate is 17.4 per 1000, which is appreciably higher than the 12.5 per 1000 reported for California in 1981.

My questions for Dr. Andrews are: (1) What screening and counseling were carried out before in vitro fertilization on these patients in order to assess perinatal risks? (2) What recommendations were made to the attending physicians regarding antepartum risk assessment, such as α-fetoprotein assessment, diabetic screening, and general pregnancy management? (3) Are there any special precautions or procedures that can be done during the in vitro fertilization procedure to reduce the well-recognized increased risk of the woman who is older than age 34 for chromosomal anomalies?

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Dr. Richard H. Paul, Los Angeles, California. I was also interested in the management approach that these unique pregnancies might require. I think you indicated that some pregnancies did reach or exceed 40 weeks and one was delivered at 42 weeks. What test or what surveillance approach would give you reassurance that everything is well in permitting a wait-and-see attitude in this very unique patient?

Dr. John T. Queenan, Washington, D. C. I was very fascinated by the 16% incidence of large for gestational age babies in your report. Additionally two of the three stillborn infants at 29 weeks were appreciably larger than we would have expected for a 29-week gestation.

Was there any explanation for the large for gestational age babies or the rapid growth in utero? Were all of these patients screened with glucose tolerance tests?

Dr. Frederick P. Zuspan, Columbus, Ohio. The rate of pregnancy-induced hypertension or hypertension in pregnancy is about 50% higher than we would anticipate nationally.

I would ask you, in the socio-economic group that this would represent, what would be the rate of pregnancy hypertension since there were probably more who had underlying chronic hypertension? Is there any way to ascertain this? It may be that this is just a spurious finding in the first hundred.

Dr. Luther M. Talbert, Chapel Hill, North Carolina. I would just like to say that we have seen the same kind of phenomena of very high cesarean section rates in the considerably smaller numbers of in vitro fertilization patients that we have delivered, mostly not in Chapel Hill. I think most of the cesarean sections we have seen have been engendered by the tremendous level of anxiety that gynecologists caring for these patients have had just because of the precious nature of the pregnancies.

I wonder if Dr. Andrews has any suggestions as to what we should say to the patient's own obstetrician when we refer her back to the community. Should we somehow discourage the performance of cesarean section in more than half of these patients?

Dr. Paul G. McDonough, Augusta, Georgia. I would like to ask Dr. Andrews about the cytogenetic findings in the studied clinical abortions. He indicated that two of the cases that they studied were "mosaics." Mosaicism may suggest a postsegmentation error during early embryogenesis. It is important, when possible, to characterize the nature and source of the cytogenetic abnormalities that occur with human in vitro fertilization. It is conceivable that these abnormalities may be quite different than those occurring in human in vivo fertilization. This type of information will help us to understand whether female meiosis II errors are more frequent than mitotic errors in early embryogenesis.

Dr. Andrews also mentioned one set of monozygotic twins in their in vitro fertilization program. The incidence of monozygotic twinning in in vitro fertilization should be monitored closely. I know of one other instance of well-documented monozygotic twinning after single-embyro transfer. An increased incidence of monozygotic twinning compared to that in vivo might suggest that technical factors in human in vitro fertilization may predispose to early blastomere separation.

Dr. Muasher (Closing). The observation by Dr. Brinkman that the pregnancy rate in women in California over the age of 34 is increasing sounded very interesting to me. Actually, when we consider patients with tubal disease who come to us for in vitro fertilization who are above the age of 40, we find no difference in the pregnancy rate after in vitro fertilization from that in patients who are under the age of 40 in our program. Furthermore, the pregnancy rate in the age group 35 to 39 is higher than the pregnancy rate in the age group less than 35.

Of course, the numbers are not statistically significant, but the observation itself I find interesting.

There were many different questions about the cesarean section rate. I think I agree with everyone that this is undoubtedly a very high cesarean rate (primary cesarean section rate), but I think it is also very understandable in view of the long history of infertility of these patients. I would also like everyone to know that many of these patients have tried in vitro fertilization with us and with other programs multiple times.

Much expense has been necessary to achieve a success, and neither they nor their obstetricians want to have any obstetric catastrophe during labor.

A very important illustration, I think, is one of the patients who had a twin pregnancy and had a vaginal delivery of the first one and a cesarean section for delivery of the other. This patient was managed by a board-certified person in maternal-fetal medicine, and after delivery of the first twin, there was an attempt to do an external podalic version to deliver the second one, as that twin was presenting as a breech.

During that attempt the fetal heart rate dropped, and there was significant vaginal bleeding. Fortunately an emergency cesarean section was performed and the baby did well, but I don't think anybody would argue that if the second baby had died, it would have been much better to perform a cesarean section in this particular case from the beginning.

As far as the high rate of perinatal mortality, I think the numbers that we presented are only for 115 babies, and I think the numbers are small to comment on that.

We usually take a very careful history from all our in vitro fertilization patients. Regarding the question about any of these patients having chronic hypertension, as far as I know, none of them did. Of the 10 patients with pregnancy-induced hypertension, seven or eight were just mild cases of pregnancy-induced hypertension. Only two cases, one ending in a stillbirth at 29 weeks and one ending in a cesarean section and delivery at 39 weeks, were severe pregnancy-induced hypertension.

We tried to suggest to the patients that they should have contemporary obstetric care, and we are in constant contact with them by telephone. However, many of these patients, as Dr. Andrews said, were delivered throughout the United States and in three foreign countries, and it is very difficult to contact their obstetricians and give them suggestions regarding what they should do during labor.

As far as the chromosomal abnormalities suggested by Dr. McDonough, we have had the opportunity to examine nine cases of abortus material, and in two of them there was an abnormality. One of the abnormalities was a true mosaic involving a trisomy 15 in one of the cell lines: 46,XX/47,XX,15+. In the other case, there was also a mosaic pattern, and in this case there was a 15-21 translocation: 46,XX/46,XX,15−,t(15,21)(p11,q11)

I am aware of some reports from Edwards and Steptoe's group in England, for example, of two cases of abortus material that had triploidy and there was one case of abortus material that had autosomal monosomy. We have not seen these in our group.

Focal vulvitis: A characteristic syndrome and cause of dyspareunia

Features, natural history, and management

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Based on the study of 67 affected women during a period of 15 years, we report the clinical features and natural history of focal vulvitis, a unique syndrome characterized by severe and persistent superficial dyspareunia and the presence of one to 11 (median three) minute, exquisitely tender areas of focal inflammation or ulceration on the mucosa of the vestibule. Three fourths of all lesions occur around the Bartholin gland ducts or between them posteriorly. Histopathologic study of tissues from seven patients has not shown a characteristic pattern of inflammation and fails to confirm a reported association between these lesions and the minor vestibular glands. These histologic studies and an in-depth clinical and epidemiologic investigation in nine patients, including microbiologic studies to identify infection by herpes simplex virus, *Neisseria gonorrhoeae, Staphylococcus aureus,* β-hemolytic streptococci, *Chlamydia trachomatis,* mycoplasmas, *Candida* sp., trichomonads, or *Mycobacterium* sp., have not established an infectious etiology for this syndrome or evidence that it represents an unusual form of an autoimmune disease or Behcet's syndrome. Treatment with topical antimicrobial or corticosteroid creams, antibiotics given systemically, or cryotherapy has not been of demonstrable benefit. Approximately one half of patients eventually experience spontaneous remission but many appear to remain symptomatic indefinitely. Surgical excision of the hymenal ring and contiguous mucosa of the vestibule has brought relief and permitted resumption of sexual activity in seven of eight treated patients. While promising, operation should be reserved for patients who have experienced unremitting dyspareunia associated with the characteristic focal inflammatory lesions for at least 6 months. (Am J Obstet Gynecol 1986;154:855-64.)

Key words: Focal vulvitis, dyspareunia, Bartholin's gland

The complaint of persistent vulvovaginal burning or discomfort, often associated with dyspareunia or pain on the insertion of tampons, usually suggests vulvovaginitis. In most patients, gross inspection and microscopic examination of vaginal discharge allows specific diagnosis and the institution of effective therapy. However, when the cause of persistent superficial dyspareunia is not obvious after thorough evaluation, particularly when there has been a history of many treatment failures, the possibility of a more rare condition, focal vulvitis, should be considered. Woodruff, Parmley, and Friedrich[1-3] have recently provided a clear description of this syndrome and attribute it to inflammation of minor vestibular glands of endodermal origin found in the vestibule. Woodruff (personal communication) has further uncovered two descriptions of this syndrome in the old literature dating from 1880[4] and 1928,[5] which indicate that it is not new. Pelisse and Hewitt,[6] in 1976, reported on 30 patients with "erythematous vulvitis in plaques" and described submucosal infiltration by chronic inflammatory cells. Others have reported similar lesions producing superficial dyspareunia.[7-9] In 1971, Dr. William Keetel described this syndrome to one of his students as "focal vulvitis" (Heuss C, personal communication), the name that we independently selected years before learning of Keetel's observations.

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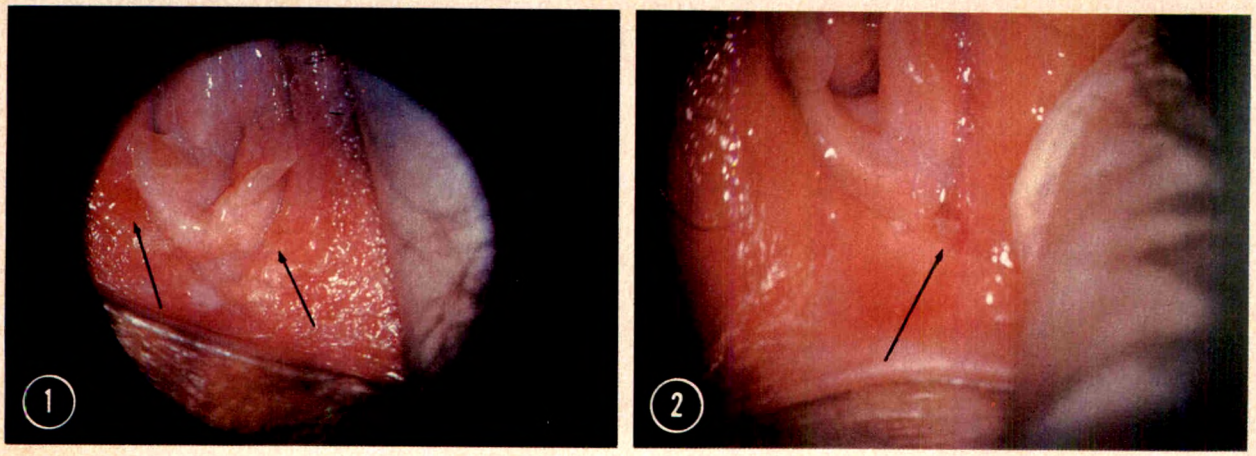


Fig. 1. The focal erythematous lesions of focal vulvitis *(arrows)* may be very inconspicuous or stand out clearly on comparison with the surrounding normal mucosa, depending on the intensity of inflammation and the character of the incident light used during examination.

Fig. 2. Focal vulvitis presenting as a minute ulcer *(arrow)*.

Until the recent reports by Woodruff et al.,[1-3] this entity had not been recognized as a distinct syndrome in the modern medical literature, and little published information has been available on its natural history or possible causes. We report clinical features, studies undertaken to identify its cause, the results of various forms of empiric therapy, and the natural history of focal vulvitis, based on the initial evaluation and longitudinal follow-up of 67 patients fulfilling criteria we propose for the syndrome, who were seen between 1971 and 1985.

Methods and results

Sources of data. One of us (B. M. P.) became aware of the syndrome in 1971, after seeing two patients whose sexual relationships had been severely impaired by excruciating superficial dyspareunia. Both had minute, focal, exquisitely sensitive inflammatory lesions on the vulvovestibular mucosa at the hymenal ring, which we have found to be highly characteristic of this condition. After repeated examinations failed to reveal evidence of vaginitis and after empiric treatment with steroidal creams and interdiction of intercourse proved ineffectual, excisional biopsy was performed to obtain tissue for histopathologic and viral studies. Cultures failed to reveal evidence of a viral cause. On microscopic examination, cells characteristic of chronic inflammation were seen in the submucosal tissues. Oral prednisone given on the possibility that the condition might represent an aberrant form of pemphigoid was also without therapeutic effect.

In 1980, two of us (B. M. P. and J. J. P.) realized that we had independently recognized this condition as a unique clinical entity and that we were both encountering increasing numbers of affected patients. It was agreed that a more formal, prospective clinical, epidemiologic, and microbiologic study should be undertaken.

All 67 patients described in this report have been examined by one or more of the authors in the University of Wisconsin Hospital Clinics (B. M. P. and D. G. M.) or in one of the School's Family Medicine Clinics (J. J. P.). Nearly all were referred for evaluation because of persistent pain and unexplained dyspareunia and others, for what was believed to represent intractable vaginitis. Nine patients have been studied in depth to an attempt to identify possible medical conditions, sexual practices, or microbial pathogens associated with this syndrome, possibly causally.

Case definition for the syndrome. Criteria included vulvar pain, dyspareunia, or pain with insertion of tampons, associated with: (1) one or more minute, exquisitely sensitive, focal inflammatory lesions located in the vestibule and (2) no identifiable cause such as herpes progenitalis, bacterial or *Candida* vaginitis, or pemphigoid.

Characteristic lesions. Casual examination of the vulva with the naked eye under room light rarely allows detection of the characteristic focal inflammatory lesions unless the examiner carefully everts the hymenal ring and exposes the inner surfaces of the labia minora. Lesions are most easily seen with a colposcope but can almost always also be seen with the naked eye with adequate exposure and a bright light source. When the hymenal ring is rolled toward the vaginal orifice with a moistened applicator, the deep crypts that lie just lateral to the ring and the urethral meatus are easily exposed and one or more exquisitely sensitive 3 to 10 mm areas of focal erythema, in most cases without swelling or ulceration, are visible (Fig. 1). Touching individual lesions with the tip of a cotton applicator (the "touch test") is essential diagnostically: The lesions

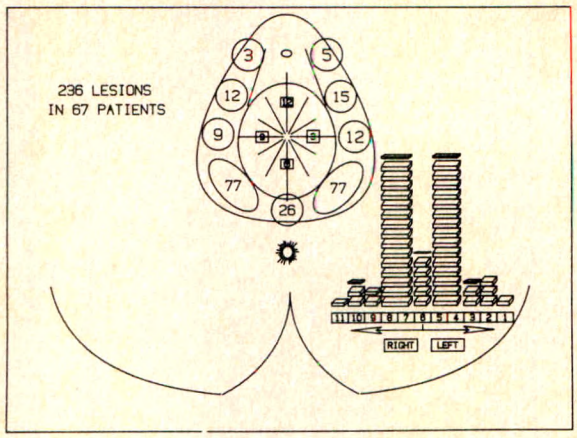


Fig. 3. The vulvar distribution of 236 lesions of focal vulvitis seen in all 67 patients after 4 and 5 o'clock and 6 and 7 o'clock positions have been combined. Each complete block represents five lesions. *Numbers inside circles* represent total number of lesions encountered at that location.

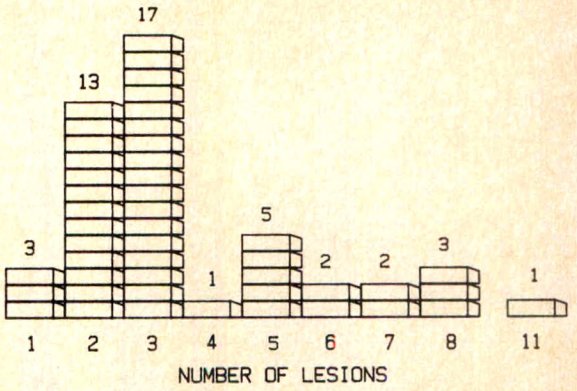


Fig. 4. The number of painful lesions per patient recorded in 47 patients with focal vulvitis evaluated by a single observer (B. M. P.).

are invariably found to be exquisitely sensitive to touch; moreover, the tenderness is characteristically very focal and can be reproduced with remarkable precision by independent observers. The contiguous mucosal surface, only millimeters from a lesion in all directions, is completely normal in appearance and nontender to touch with the applicator.

Fifteen of our 67 patients (22%) showed minute discrete ulcers in the mucosa at one or more of the sites of inflammation and focal tenderness (Fig. 2). In some patients, the mucosa was superficially denuded or appeared split. In others, tiny indolent ulcers could be seen deep within the crypts, out of sight unless the area was fully exposed (Fig. 2).

Location of lesions. Lesions in each patient were mapped diagramatically (Fig. 3). Lesions were most commonly located in the area about or posteriorly between the Bartholin duct openings between 4 and 8 o'clock. (The duct orifices, however, are usually not visible to the naked eye and even through the colposcope can be identified only occasionally.) In this location, inflammatory lesions were usually largest, often measuring from 8 to 10 mm in diameter. Evidence of associated inflammation or especially suppurative infection of the Bartholin glands, however, has not been seen. When lesions occur at 6 o'clock as well, the mucosa of the vestibule between 4 and 8 o'clock may be diffusely erythematous. There is seldom a well-formed crypt in the 6 o'clock position and, when it is involved, redness is more diffuse and may even extend onto the surface of the perineal body.

When lesions at 4 and 5 o'clock and at 7 and 8 o'clock are combined (Fig. 3), these locations account for 65% of all lesions; when the lesions at 6 o'clock are added, 76% of all lesions occur posteriorly, between the 4 and 8 o'clock positions. In 54 patients (81% of the total group), all of the lesions identified were located on the mucosa of the vestibule between 3 and 9 o'clock inclusive.

Number of lesions. Among 47 patients seen by B. M. P., the number of sites per patient varied from one to 11 (median three). Patients with either two lesions (13 patients) or three lesions (17 patients) accounted for 64% of the affected patients (Fig. 4).

Initial clinical presentation and features of patients. The features of these patients and the vulvitis are summarized in Table I. All were Caucasian. The age at the onset of the syndrome varied from 14 to 67 (median 25) years (Fig. 5); 49% had the onset in their 20s. Fifty-one percent had been pregnant; approximately one fourth had had a spontaneous or induced abortion.

The records of 50 patients provide reasonably detailed information concerning the character of the syndrome's onset. In 39 patients (80%), the onset was described as sudden and first noted during a specific intercourse. Eleven patients (20%) reported a more gradual onset, occurring over a period of weeks or months. In 18 (36%) of these 50 patients, their physicians had ascribed their symptoms to vaginitis, most often *Candida* vaginitis. However, even when there was microscopic evidence of vaginitis during our initial examination, successful treatment of the vaginitis did not result in resolution of the lesions or relief of symptoms.

In six patients, the characteristic pain was experienced with the first attempt at intercourse; one of these patients had been experiencing pain with the insertion of tampons for several years previously. With the exception of these six patients, all of the patients had enjoyed pain-free intercourse with one or more partners for months or years prior to the onset of focal vulvitis. Once vulvitis occurred, however, most of these women markedly curtailed the frequency of sexual intercourse and many had become essentially abstinent.

Four other patients indicated that they had first experienced pain when inserting tampons, and two be-

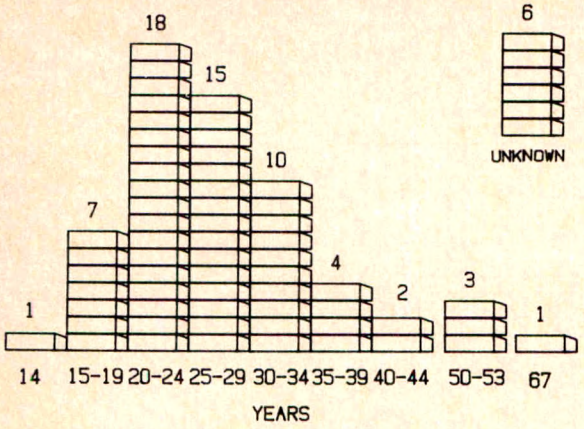


Fig. 5. Age distribution of 67 patients at the onset of symptomatic focal vulvitis.

lieved that the tampon inserter was responsible for their symptoms.

Although many of these patients had had urinary tract infections in the past, urinary tract symptoms or evidence of active infection has not been associated with this syndrome. Recurrent symptomatic cystitis or urethritis was reported by only two of these patients.

A number of the women, after repeated negative examinations, had been referred for psychiatric counseling but had continued to experience incapacitating dyspareunia.

The length of time symptoms had been present at the time of our initial evaluation varied from a few months to 26 years (Table I). Nearly half the patients had been symptomatic for ≥2 years.

In-depth study of nine patients. In 1981, when it seemed clear that many patients presenting with dyspareunia had focal lesions producing a characteristic syndrome, nine patients fitting the case definition were prospectively evaluated in the University Hospital Clinics for factors associated with the syndrome, in an effort to seek a cause. This evaulation consisted of a detailed sexual history (Table II), a comprehensive medical evaluation, pelvic examination with colposcopic photography, laboratory studies (hemogram, erythrocyte sedimentation rate, 12-test biochemical profile, tests for rheumatoid factor and antinuclear antibodies, IgG, IgA, IgM, and IgE levels, and urinalysis), microscopic studies for evidence of *Candida, Trichomonas,* and nonspecific vaginitis, and vaginal cultures to detect colonization by β-hemolytic streptococci, *Staphylococcus aureus, Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma* sp., herpes simplex virus, and *Mycobacterium* sp.[10]

No intercurrent conditions, allergies, family history of gynecologic diseases, or extragenital physical findings emerged in a frequency that could be considered significant, and none of the nine women reported knowledge of a first- or second-order relative with similar symptoms. Moreover, except for mild anemia in one patient and subnormal levels of IgA in another, the screening laboratory studies, including absolute granulocyte and lymphocyte counts, sedimentation rate, and immunoglobulin levels, were uniformly in a normal range in all nine patients. None of the patients showed any clinical or laboratory evidence of constitutional illness or an autoimmune disorder.

Table I. Features of patients with focal vulvitis

Feature	*No.**	*%*
Age at onset (yr) (median and range)	25 (14-67)	
Race		
Caucasian	67	100
Black	0	
Asian	0	
Parity		
Gravida 0	30	49
Primiparous	7	11
Multiparous	8	13
Prior abortions	16	26
Onset of vulvitis		
Acute	39	80
Gradual	11	20
Duration of symptoms at first visit (yr)		
>1.0	26	41
1-1.9	11	17
2-26.7	26	41

*The relative frequencies of various factors are based on different numbers of patients for each factor for which complete data are available.

The results of the detailed sexual history and other relevant historical information are shown in Table II. The menstrual history was unremarkable in all nine patients, and no single form of birth control predominated. Four of the women had used a vaginal douche but none had used commercial vulvar sprays or perfumes before onset of the syndrome.

The age at first intercourse ranged from 19 to 27 years and the group had been sexually active for an average of 7 years before becoming symptomatic; two women, however, had experienced characteristic symptoms with first intercourse and had continued thereafter to suffer from dyspareunia. Half of the group had had five or more sexual partners, and six (60%) had acquired a new partner in the 6 months before onset. Nearly all had engaged in cunnilingus and fellatio, but only one had engaged in anal intercourse. Eight of the nine women had masturbated at variable frequency before the onset of vulvitis. None had had any homosexual experience.

Most notably, the frequency of intercourse fell precipitately with the onset of vulvitis, from a median of four times per week before onset of symptoms to far less than once per week after they began to experience dyspareunia; five women had become abstinent.

Table II. Sexual history and other medical history before onset in nine patients with syndrome of focal vulvitis with dyspareunia

Feature	*No.*	*%*
Menstrual history		
Menarche (yr) (median and range)	12 (10-17)	
Normal menses	9	100
Tampon use	9	100
Birth control		
Oral contraceptives	4	44
Intrauterine device	1	11
Diaphragm	3	33
Condoms	1	11
Douching	3	33
Use of vaginal sprays or perfumes	0	
Sexual history		
Age of first intercourse (yr) (median and range)	20 (19-23)	
How long sexually active before onset (yr) (median and range)	7 (0-21)	
No. of lifetime sexual partners (mean and range)	5 (1-30)	
Frequency of intercourse per week (mean and range)		
Before onset	4 (1-10)	
Since onset	<1 (5 abstinent)	
Oral-genital practices		
Cunnilingus	8	89
Fellatio	8	89
Anal penetration	1	11
Use of artificial lubricant	4	44
Masturbation	8	89
Homosexual activity	0	
History of sexually transmitted disease		
Gonorrhea or syphilis	0	
Herpes progenitalis	0	
Nonspecific urethritis	1	9
Condyloma acuminatum	2	22
History of prior urinary tract infection	6	67
History of vaginitis	7	78
Candida	6	67
Unknown	1	11
History of oral lesions		
Aphthous ulcers	4	44
Herpes labialis	2	22
Sexual partner		
History of genital lesions	1	9
Oral aphthous ulcers	3	33
Oral herpes labialis	0	
Character of vulvitis		
Age of onset (yr) (median and range)	26 (19-38)	
Length of time symptomatic (mo) (median and range)	24 (4-48)	
Onset		
Acute	5	55
Gradual	4	44

Few had had a sexually transmissible disease before becoming symptomatic and, although two thirds had been treated for one or several bouts of symptomatic urinary tract infection, none experienced dysuria or other irritative urinary symptoms in association with vulvitis. Although three fourths reported being treated for vaginitis prior to developing dyspareunia, none related symptoms consistent with vaginitis or showed evidence of *Candida*, *Trichomonas*, or nonspecific vaginitis on pelvic examination or microscopic study at the time of our initial evaluation.

Table III. Results of local cryotherapy in 21 patients with focal vulvitis compared with 43 untreated patients*

	Cryotherapy		*Untreated*	
	n	*%*	*n*	*%*
No. of patients	21	100	43	100
Results of therapy				
Remission	8	38	16	37
Improvement	2	10	7	16
Unimproved	11	52	20	47

*None of these differences are statistically significant at $p < 0.05$.

Less than half the patients related a history of recurrent aphthous stomatitis ("canker sores") or experiencing recurrent "cold sores" consistent with herpes labialis. Even fewer related a history of recurrent oral or labial lesions in a sexual partner.

Combining the results of the microbiologic studies done in these nine patients with studies done on a sporadic basis in the other 58 patients shows that, of 11 patients tested, all had negative cultures for *Neisseria gonorrhoeae*; of seven patients tested for colonization by *Chlamydia trachomatis*, all had negative cultures; 13 (50%) of 26 patients screened had positive cultures for mycoplasmas, usually *Ureaplasma urealyticum*; and all 14 tested had negative cultures for herpes simplex virus. None of the nine women prospectively studied was found to be a vaginal carrier of β-hemolytic streptococci, *S. aureus*, or *Mycobacterium* sp.

Treatment. After our initial examination, patients were managed in one of three ways: (1) followed up without specific therapy other than treatment of unrelated medical problems, (2) treated with local cryotherapy with the goal of destroying the lesions, or (3) in selected cases, beginning in 1984, treated by surgical excision of the hymenal ring and contiguous vestibular mucosa and submucosa as described by Woodruff and Parmley.[1]

Nonspecific therapy. Before our initial evaluation, most of these patients had been treated with a variety of vaginal creams or systemic antibiotics, most often tetracyclines, ampicillin, or metronidazole, usually for suspected vaginitis, without experiencing relief of their symptoms. Nine patients had received a 7- to 10-day course of a tetracycline, prescribed in six patients because of recovery of *Mycoplasma* sp. in culture and in three, as early empiric therapy of the characteristic syn-

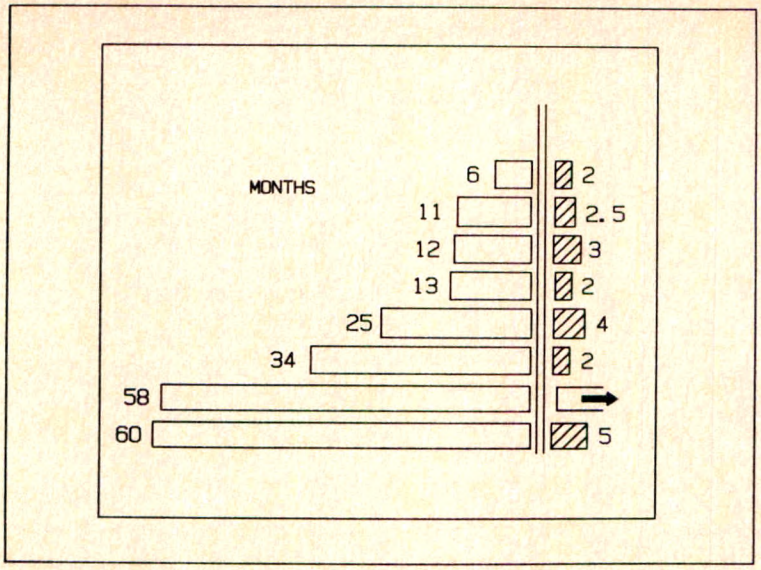


Fig. 6. Duration of symptomatic focal vulvitis before operation and length of disability after operation. In one of the eight patients, symptoms have continued unabated.

drome. Three of the nine patients experienced remission of their symptoms within 3 to 4 months, and one, 6 months later; two reported moderate improvement after treatment; the remaining four patients had no improvement. These data, similar to the natural history of the syndrome in untreated patients, do not suggest significant therapeutic benefit from the use of tetracyclines.

Cryotherapy. Twenty-two patients seen initially by one of us (J. J. P.) were treated with local cryotherapy aimed at destroying the focal areas of inflammation. None of the 45 patients managed by B. M. P. received cryotherapy. Comparison of the two contemporaneously managed groups reveals no significant differences in age at onset of the syndrome, parity, distribution of lesions, or duration of vulvitis.

Three patients did not return after their first visit and were lost to follow-up. Twenty-one evaluable patients received one to five (mean two) cryotherapy treatments of lesions and 43, no cryotherapy (Table III).

Among those treated with cryotherapy, eight (38%) became completely asymptomatic, two improved to the degree that they no longer felt the need for further treatment, and 11 (52%) remained unimproved. Of the eight that became asymptomatic, remission occurred in four during the first 6 months after therapy; in the remaining four, remission occurred more than 6 months later.

In the 43 untreated patients, 16 (37%) eventually had a complete spontaneous remission, seven (16%) reported partial improvement, and 20 (47%) remained unimproved. These results indicate that cryotherapy did not significantly affect the course of the disease.

Surgical excision. In 1983, Woodruff and Parmley[1] reported that surgical perineoplasty, consisting of resection of the hymenal ring and contiguous 0.5 cm of mucosa and submucosa had successfully alleviated the severe dyspareunia associated with this syndrome in 14 of 15 women operated on. To date nine of our patients have undergone this procedure. The results affirm the promising results of Woodruff and Parmley. Of the eight patients who have been followed up long enough for meaningful assessment of the therapeutic outcome (Fig. 6), seven who had been symptomatic from 6 to 60 months before operation are now asymptomatic and sexually active. One patient, symptomatic for 58 months before operation, remains a surgical treatment failure and is unable to have sexual relations without pain. One patient experiences occasional brief discomfort at intromission. The period of disability that follows successful operation, however, has never been less than 2 months and has lasted for as long as 5 months (Fig. 6).

Course of the disease. At the time of last contact, symptoms of the disease had persisted in 34 (51%) of the 67 patients. Twenty-four (36%) are in complete remission and nine (13%) have improved to the point that they no longer are seeking definitive therapy.

In two patients with continuing disease, the date of onset of symptoms is not known. The duration of symptoms for the remaining 32 patients ranges from 2 months to more than 18 years (Fig. 7). The severity of symptoms fluctuates. Discomfort is often worse in the week before menses. Some women have intermittent vulvar burning, which is precipitated or exacerbated by tight clothing.

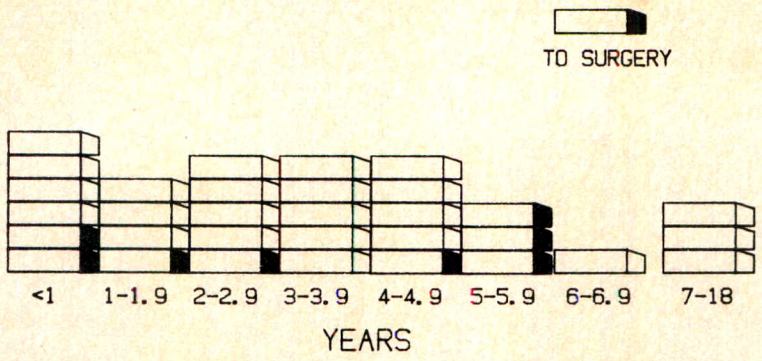


Fig. 7. Duration of symptoms at the time of last contact or the date of operation in 32 patients with continued focal vulvitis with dyspareunia.

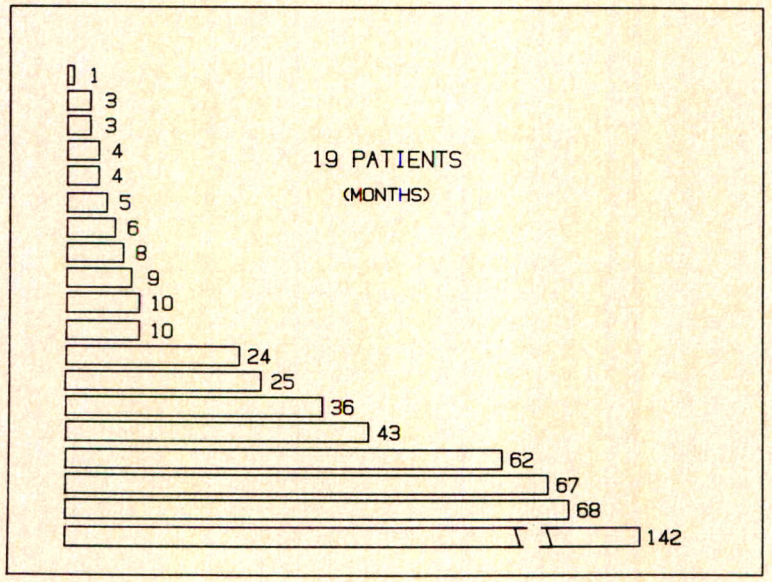


Fig. 8. Duration of symptoms of focal vulvitis at the time of complete spontaneous remission in 19 of 24 patients. In five patients complete data were not available.

Of the 24 women in complete spontaneous remission at the time of last contact, the date of onset of symptoms or the date of onset of remission is not available in five. In six of the remaining 19, the duration of symptoms at the time of remission was less than 6 months and remission was very rapid from its outset (Fig. 8).

In the remaining 13 patients now in complete remission, lesions and symptoms had persisted from 6 months to 12 years before remission. In most, improvement before complete resolution of all discomfort occurred only very gradually and has been difficult to time accurately.

Several patients who are free of pain at times other than intromission have learned to anesthetize the lesions with 4% aqueous Xylocaine with the aid of their sexual partners and enjoy comfortable intercourse. It is important that the Xylocaine be applied precisely, only to the painful lesions, with individual cotton-tipped applicators. These will be held in place automatically by the labia if the patient brings her legs together after the application. On removal of the applicators after 3 or 4 minutes, pain-free intercourse is usually possible for at least 10 to 15 minutes.

Pathologic findings. The surgical specimens from seven patients operated on at this institution were completely blocked for sectioning. In six cases, it was possible to accurately approximate the positin of each block on the vulva.

Unlike Robboy et al.,[11] who counted only those glands located "anterior to the vagina," we have counted all superficially located mucinous glands regardless of their position about the opening of the vagina (Fig. 9). Glands were found "anteriorly" (10 and 11 o'clock and 1 and 2 o'clock) in only two of the seven specimens. Posteriorly (3 through 9 o'clock), similar small glands were found singly or in multiples on one or both sides

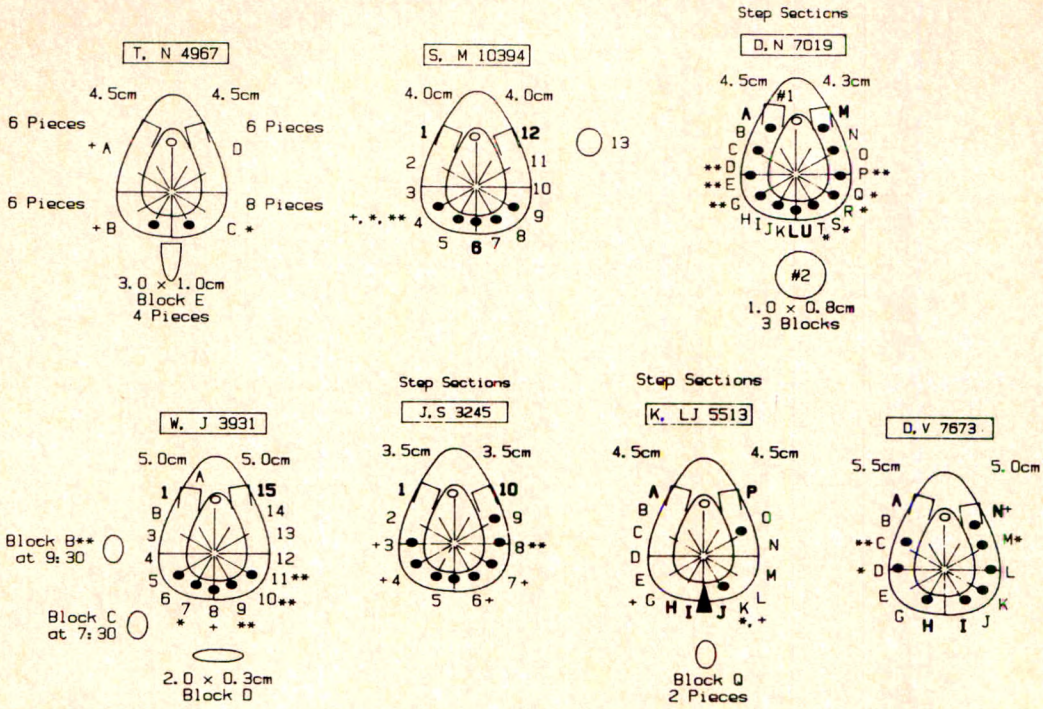


Fig. 9. The relationship of pathologic findings in serially blocked surgical specimens to vulvar lesions identified clinically. In each instance, the first block (designated as *A* or *1*) was taken from the apex of the arm of the Y- or U-shaped specimen on the patient's right. The positions of subsequent serial blocks, ascertained from the pathologic description, are indicated diagrammatically. When the exact location of an individual block is known, its position is indicated in *boldface type*. The remaining blocks have been assumed to be of equal size and have been distributed evenly between these known locations. To achieve a more complete sampling of the tissues, the original blocks from three specimens were reembedded in groups and were step sectioned after the routine sections had been made. The positions of the original lesions as identified at the initial examination are also diagrammatically displayed for each patient. * = Mucinous glands. ** = Mucinous glands associated with lymphocytic infiltration. + = Submucosal lymphocytic infiltration.

in all seven specimens examined; when many glands were found, the most common locations were between 3 and 5 o'clock and 7 and 9 o'clock. What appeared to be the opening of the duct of the major (Bartholin) gland was seen in the vicinity of these glands in only two specimens. No glands were seen in the area of the fourchette or perineal skin (6 o'clock) in any of the specimens.

Whether found singly or in groups, the numbers of inflammatory cells about these glands were not impressive (Fig. 9). Infiltration of the periglandular tissues by lymphocytes, occasionally associated with small numbers of plasma cells, was identified in five of the seven patients but never around all of the glands seen in the section.

Focal submucosal collections of lymphocytes unassociated with glands were seen in five of the seven specimens but were an impressive feature in only one. The epithelium over the most intensely infiltrated areas was usually thin and without papillary pegs. Perivascular infiltration by inflammatory cells, suggestive of vasculitis, was not seen in any of the sections examined.

The correlation between the locations of painful focal lesions and the sites of glands or focal infiltration by inflammatory cells on histologic examination of vulvar tissues from these patients is poor (Fig. 9).

Comment

We report a form of vulvitis characterized by severe and persistent superficial dyspareunia and the finding of one to 11 (median three) exquisitely tender, 3 to 10 mm areas of focal inflammation, with or without superficial ulcers, located on the mucosa of the vestibule. Three fourths of the lesions in 67 patients were located around the openings of the Bartholin ducts or between them posteriorly (Fig. 5), and 80% of the patients had lesions confined to this area. Based on our experience with 67 patients encountered during a 15-year period, we believe that this is a common cause of persistent, severe, superficial dyspareunia. Although adult women of all ages appear to be susceptible, the majority of cases have occurred in women in their 20s. Virtually all experience severe introital pain with intercourse; many have as a consequence become abstinent.

We believe that these patients are suffering from a condition described many years ago by several early observers,[4] including Kelly,[5] and independently rediscovered only recently by Woodruff and Parmley,[1] Friedrich,[2] and ourselves. We think the term focal vulvitis most simply and accurately describes the syndrome.

Woodruff and Parmley[1] and Friedrich[2] have recently provided a histopathologic description of these lesions, reporting infiltration about the minor vestibular glands of the vestibule by cells characteristic of chronic inflammation. In a careful histologic study of the surgical specimens from seven patients who were operated on (Fig. 9), we have counted all superficially located glands, regardless of their position about the opening of the vagina, by examining multiple sections from each of serial blocks of tissue. Neither these glands nor the focal areas of chronic inflammatory cells correspond closely with the lesions that were present in any given patient (Fig. 9). Moreover, the small numbers of lymphocytes seen about the glands in five of the seven specimens are not considered sufficient to indicate clear-cut glandular inflammation except in one patient.

Our failure to find large numbers of plasma cells in the hundreds of tissue sections examined would seem to exclude the possibility that the lesions we have described are the same as those described by Zoon[12] in men and designated "balanitis circumscripta plasmacellularis" and by others in women under a variety of names[13, 14]; the main distinguishing histopathologic feature of the lesions described in these reports is infiltration by large numbers of plasma cells.

We agree, however, with Woodruff and Parmley[1] that the painful lesions correlate best with what is probably endodermally derived mucosa, with or without glands, wherever it exists in the vestibule. Woodruff and Parmley[1] suggest that, with individual variation, only a portion of the epithelium covering the vestibule is of endodermal origin, that covering the fourchette and the inner surfaces of the labia minora, and offer as support the extreme sensitivity of the epithelium in these areas to 5-fluorouracil used in the treatment of vaginal carcinoma in situ. We have also noted, when using 5-fluorouracil in the vagina, that the most severe reaction occurs posteriorly, between 4 and 8 o'clock, and the least severe, anteriorly, between 9 and 3 o'clock, suggesting a smaller proportion of sensitive endodermally derived epithelium in these areas. Less endodermally derived epithelium in these areas might explain the infrequency of lesions in these areas (Fig. 3) as well as the relative rarity of minor vestibular glands between 10 and 2 o'clock as noted by us (Fig. 9) and reported by Robboy et al.[11] Epithelial primacy in pathogenesis might also explain the high frequency of lesions seen at 6 o'clock and their more diffuse character and the absence of vestibular glands seen in tissue sections from this area.

The relatively minimal histologic findings we report and others have described do not point toward any clear-cut cause. The normal appearance of the vagina, the clinical features of the lesions, and the negative microbiologic studies make it unlikely that this is an unusual form of vaginitis or an infection caused by herpes simplex virus, *N. gonorrhoeae*, *S. aureus*, β-hemolytic streptococci, *C. trachomatis*, mycoplasmas, or *Mycobacterium* sp. involving the mucosa, the submucosa, or the minor vestibular glands. The 50% rate of vaginal carriage of mycoplasmas in 26 of the women tested is comparable to carriage rates reported in asymptomatic, healthy, sexually active young women.[16] Moreover, we have been unable to obtain a history from any of our patients, including the nine studied in depth, to suggest that this is a transmissible disease; none of the women related a history of similar genital lesions in a sexual partner. And no evidence could be adduced that antimicrobial therapy received by many of these women, with a drug or drugs effective against the common agents of vaginitis, or against mycoplasmas or *C. trachomatis*, was of any demonstrable therapeutic benefit.

Based on the detailed sexual history obtained from nine of the women (Table II) it might be assumed that women developing this syndrome are sexually more active in terms of number of lifetime partners and oral-genital practices, but six of the 67 patients had the onset of this syndrome with first sexual intercourse. Future studies should include a case-control comparison of sexual practices in a large number of patients with the syndrome and asymptomatic control patients, matched for age, parity, and socioeconomic status.

It seems clear that this is not a manifestation of a systemic disease such as an autoimmune disorder or a vasculitis. It is possible that the cause will continue to be elusive, as has been the case with recurrent aphthous stomatitis.[17] Clearly, patients with focal vulvitis do not experience oral aphthous ulcers or herpes labialis more frequently than the population at large, nor do their sexual partners (Table II). Moreover, none of our patients have gone on to develop the classic constellation of oral-genital ulcers, inflammatory ophthalmologic lesions, and neurological disease characteristic of Behcet's syndrome.[18]

Given the nonspecific histopathologic findings and the negative results of studies to identify an infectious or autoimmune etiology, the condition we describe must continue to be regarded as a syndrome. Accordingly, with the lack of a reliable diagnostic laboratory test or characteristic histopathologic features, there is some hazard of misdiagnosis by the clinician. However, if other causes of acquired superficial dyspareunia such as hymenal tears, mucosal splits or abrasions (which

usually heal rapidly), Bartholin's cyst or abscess, herpes progenitalis, and vaginitis are excluded, if severe dyspareunia has persisted for a number of months, and if the characteristic exquisitely sensitive, highly focal lesions in the posterior vestibule have been clearly and repeatedly identified, we believe this syndrome can be diagnosed quite accurately.

The patient can aid in establishing the diagnosis by confirming the very focal nature of the lesions. If she can be taught to reliably identify and locate the lesions with a mirror or the aid of her partner and to anesthetize them locally with 4% Xylocaine, comfortable sexual relations should be possible.

Our early attempts to cure this syndrome with local therapy, including cryotherapy, have been unsuccessful. However, the highly focal nature of these lesions should encourage further investigation of alternative methods of local ablation with the goal of avoiding the expense, risks, and inconvenience of surgical excision, as described by Woodruff and Parmley,[1] which promises to provide definitive relief in most cases. Laser therapy appears to have been successful in patients with superficial dyspareunia when focal lesions are few and well localized.[15] The surgical procedure described by Munsick,[8, 9] which has been used for a variety of conditions causing dyspareunia, may well be of value for some patients with focal vulvitis but, like laser therapy, should also be carefully studied. We think that it is very likely that some of the patients Munsick has treated have been patients with unrecognized focal vulvitis.

Approximately one half of patients with focal vulvitis will eventually experience spontaneous improvement, most often total remission, and in about one half of these patients this occurs within 6 months; thereafter the probability of remission for patients who remain symptomatic falls to <25%, and it appears that many of these patients will remain symptomatic indefinitely (Fig. 8). Surgical perineoplasty as described by Woodruff and Parmley[1] appears to be effective in most cases, but in our judgment should be reserved for those patients who have experienced unremitting dyspareunia of unknown cause associated with the characteristic focal inflammatory lesions for at least 6 months.

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Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease

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Fifty-five women with suspected pelvic inflammatory disease underwent diagnostic laparoscopy and endometrial and tubal biopsy, with specimens for isolation of *Chlamydia trachomatis* and for staining with a species-specific monoclonal fluorescein-conjugated antibody to *C. trachomatis* were obtained from the urethra, rectum, endocervix, endometrium, tubes, and cul-de-sac. *C. trachomatis* was isolated from 21 patients (38%), including 10 (18%) who had positive endometrial or tubal cultures. The fluorescein-conjugated antibody stain was positive for 43 (86%) of 50 culture-positive specimens, for 14 (18%) of 78 culture-negative specimens from 21 patients who had positive cultures from other sites, and for one (0.5%) of 192 specimens from 34 patients who had negative cultures at all sites. Thus the sensitivity of direct fluorescein-conjugated antibody for culture-positive specimens was 86% and the specificity for specimens from culture-negative patients was 99%. Twelve upper genital tract specimens were positive by fluorescein-conjugated antibody only. Fluorescein-conjugated antibody staining of 50 paraffin-embedded endometrial aspirates showed extracellular or intracellular elementary bodies and or cytoplasmic inclusions in all of seven culture-positive specimens, in four of six culture-negative specimens from patients who had positive cultures at other sites, and in none of 34 specimens from patients with negative cultures. Thus fluorescein-conjugated antibody staining is useful for confirming the role of *C. trachomatis* in endometritis and salpingitis. It is more sensitive than culture for detection of chlamydia in endometrial or tubal specimens and is able to confirm that the organism is actually present in endometrial tissue (rather than simply reflecting contamination from the cervix) in women with clinical evidence of pelvic inflammatory disease. (Am J Obstet Gynecol 1986;154:865-73.)

Key words: Diagnosis of *Chlamydia trachomatis,* pelvic inflammatory disease, monoclonal antibodies

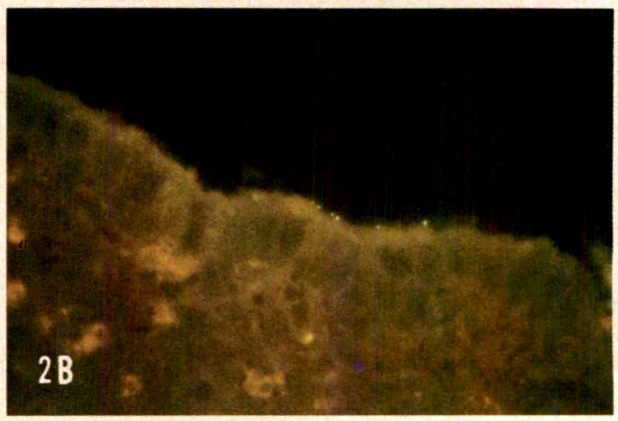
C. trachomatis is now recognized as a leading cause of pelvic inflammatory disease.[1] However, diagnosis of chlamydial infection by culture is time-consuming and expensive, and methods for isolation in tissue cell culture are often unavailable. In addition, distinguishing endometrial infection from endocervical infection is confusing even when endometrial biopsy tissue is obtained for culture, because of possible contamination from the endocervix or vagina that may occur while endometrial samples are obtained. Among patients attending sexually transmitted disease clinics or family planning clinics, direct immunofluorescence has recently been shown to compare favorably with culture for diagnosis of genital chlamydial infection. Direct immunofluorescence staining of endocervical specimens, with a fluorescein-conjugated monoclonal antibody to *C. trachomatis,* had a sensitivity of 86% to 93% and a specificity of 93% to 99% as compared with those of isolation of the organism in tissue cell culture.[2, 3] To investigate the use of direct immunofluorescence for diagnosis of chlamydial infection in women with suspected PID, and to see if the method would allow us to more firmly establish a diagnosis of chlamydial endometritis and salpingitis, we collected multiple samples from upper and lower genital sites, as well as endometrial tissue biopsies, for culture and for immunofluorescent staining.

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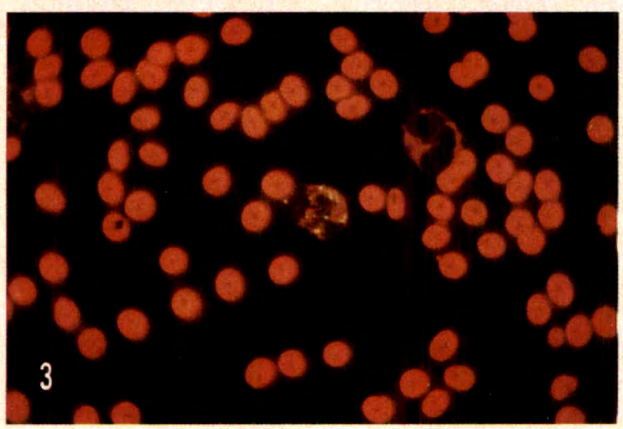


Fig. 1. Direct immunofluorescent staining of cervical smears with fluorescein-conjugated monoclonal antibodies to *C. trachomatis*. *A*, Note small round extracellular elementary bodies of *C. trachomatis*. (Original magnification ×500). *B*, Note intracellular inclusion body of *C. trachomatis* in an exfoliated endocervical cell. (Original magnification ×500).

Fig. 2. Paraffin-embedded endometrial biopsy tissue stained with fluorescein-conjugated monoclonal antibody to *C. trachomatis*. *A*, Note inclusions of *C. trachomatis* within the surface glandular epithelial cells. *B*, Extracellular elementary bodies of *C. trachomatis* adherent to surface glandular endometrial cells.

Fig. 3. Direct immunofluorescent stain of endosalpingeal smear obtained at laparoscopy, showing elementary bodies of *C. trachomatis* associated with a polymorphonuclear leukocyte.

Material and methods

Study population. The study population included 55 consecutively studied women with lower abdominal pain and adnexal tenderness, presenting to the sexually transmitted disease clinic, emergency room or women's clinic at the Harborview Medical Center or the emergency room or women's clinic at the University Hospital. None had received antibiotics in the preceding 14 days.

Diagnosis of endometritis and salpingitis. A lapa-

roscopic diagnosis of acute salpingitis was made if there was erythema and edema of the fallopian tubes and either purulent exudate, fibrinous exudate, or fibrinous adhesions that could be readily broken. Diagnosis of endometritis was based on presence of at least 10 plasma cells per 120× field of endometrial tissue present.

Serologic studies. Serum was obtained on enrollment and all patients were asked to return at 2, 4-6, and 12 weeks after enrollment when serum was again obtained for analysis of acute and convalescent serum microimmunofluorescent total antibody and immunoglobulin M antibody to *C. trachomatis*. Acute sera and convalescent sera obtained 2 to 6 weeks after initial examination were available for testing from 39 patients.

Microbiologic studies

Specimen collection. Samples were obtained from the endocervix, urethra, rectum, endometrium, fallopian tubes, and cul-de-sac for culture and for fluorescent antibody staining for chlamydia. Endocervical cultures were obtained after the ectocervix had been wiped clear of vaginal discharge with large swabs. A type III calcium alginate swab or a cotton swab on a plastic shaft (Inolex, Glenwood, Illinois), from lots that were shown not to inhibit *C. trachomatis* growth in vitro, was introduced into the endocervical canal to obtain material for *C. trachomatis*. After the swab was rotated several times in the endocervical canal, it was placed in 1 ml of sucrose-phosphate chlamydia transport medium containing 2% fetal calf serum, 100,000 units of penicillinase, and 1 μg of gentamicin, 25 μg of vancomycin, and 25 units of nystatin. A second cotton-tipped swab was inserted and rotated in a similar manner and then used to smear endocervical material onto a glass slide, which was immediately fixed. Slides from the first 24 patients were fixed in 95% ethanol while slides from the last 31 patients were fixed in acetone (no difference in quality of staining related to these two fixatives was noted). An endocervical scrape was obtained with the rounded end of a wooden Ayre's spatula, and this material was likewise smeared onto a glass slide and placed in 95% ethanol. Rectal and urethral specimens were collected for *C. trachomatis* cultures and for slides for fluorescent antibody staining.

Under general anesthesia, patients then underwent endometrial aspiration and biopsy with a Novak curette. The endocervix was first prepared with a Betadine-alcohol swab. Endometrial material was aspirated through a Novak curette with a 10 ml syringe. Care was taken not to aspirate into the endocervical canal during removal of the curette. One drop of the curetted endometrial material was inoculated into transport media for culture for *C. trachomatis*. A second drop of material was smeared onto a glass slide and immediately fixed in 95% ethanol for up to 24 hours. The remainder of the endometrial tissue specimens were then fixed in buffered neutral formalin for histopathologic studies.

A laparoscopic examination was next performed, at which time any fluid present in the cul-de-sac was aspirated. One portion of the fluid was sent for culture for *C. trachomatis*, and the remainder was fixed in an equal amount of 95% ethanol or acetone for fluorescent antibody staining. If fallopian tubes were patent, an aluminum shaft calcium alginate swab was inserted through the laparoscope and introduced through the ostia 1 to 2 cm into the fallopian tubes. Material from the swabs was smeared onto slides for fluorescent antibody staining, as described above, and was also submitted for culture. If neither tube was patent a tube was punctured with an 18-gauge spinal needle and material was aspirated for culture and fluorescent antibody staining. Fimbrial biopsies were performed with a flexible esophageal pediatric biopsy forceps (Olympus Co., Tokyo) for histologic examination and fluorescein-conjugated antibody staining.

Isolation of C. trachomatis. Each specimen was inoculated into three wells of a 96-well microtiter plate as well as into two vials (one of which was used for secondary passage) containing cycloheximide-treated McCoy cells.[4] After 48 hours, cell layers were stained with fluorescein-conjugated monoclonal antibodies and examined for chlamydial inclusions.[5]

Fluorescent antibody staining of smears and tissue. Slides were removed from ethanol or acetone within 24 hours and if not immediately stained were frozen at −70° C for 1 to 2 weeks. Slides were stained as previously described[6] with fluorescein-conjugated monoclonal antibody to *C. trachomatis* (Syva Corp., Palo Alto, California). This antibody to the major outer membrane protein with a molecular weight of 40,000 dalton reacts with all 15 known serovars of *C. trachomatis* but not with *Chlamydia psittaci*. In 25 cases, 150 μl of solution containing antibody was applied to each slide. Slides were then incubated at room temperature for 30 minutes. In the remaining 30 cases, 100 μl of solution containing antibody was applied to each slide and the slides were then incubated for 15 minutes at room temperature. After removal of excess antibody, the slides were rinsed with ionized water and air dried. A combination of Tris buffer and glycerol was used as mounting medium and coverslips were placed on the slides. Slides were examined immediately without knowledge of other clinical, histologic, cytologic, or microbiologic findings. Specimens were examined with an 8× ocular lens and a 40× objective lens. The presence of elementary bodies of *C. trachomatis* and of typical chlamydial inclusions were confirmed with a 63× oil immersion lens. The location of elementary bodies (within inflammatory cells, within epithelial cells, and/or cell-free or at-

Table I. Results of culture and direct immunofluorescent staining for *C. trachomatis* by site tested among women with positive results at any site by either method

	*Results of culture/results of direct immunofluorescent stain**						
Patient code	*Urethra*	*Rectum*	*Cervix*	*Endometrium*	*Left tube*	*Right tube*	*Cul-de-sac*
PO2	−/−	−/−	+/+	−/−	−/−	−/−	−/−
PO9	−/−	−/−	−/−	−/−	−/+	−/−	−/−
PO10	−/ND†	+/+	−/−	+/+	+/+	ND	−/−
PO12	+/−	−/ND	−/−	−/−	−/−	−/−	−/−
PO16	+/+	ND	+/+	−/+	−/+	+/+	−/−
PO19	+/+	+/−	+/+	−/+	−/−	−/−	−/−
PO20	+/−	−/−	+/−	+/+	−/ND	−/ND	−/−
PO22	−/+	+/+	−/+	−/+	−/+	ND	−/+
PO25	−/−	−/−	+/+	+/+	−/−	−/−	−/−
PO26	−/−	+/−	−/+	−/+	−/+	−/+	−/−
PO27	+/−	+/+	+/+	−/−	ND	+/+	−/−
PO28	+/+	−/−	+/+	+/+	+/+	−/−	−/−
PO29	+/+	+/+	+/+	+/+	−/−	−/−	−/−
ICD3	−/−	−/−	+/+	−/−	−/−	−/−	−/−
ICD5	+/+	+/+	+/+	+/+	−/+	ND	+/+
ICD9	+/+	+/+	+/+	−/+	+/+	ND	−/−
ICD10	+/−	−/−	+/+	−/−	−/−	−/−	−/−
ICD14	−/−	−/−	+/−	−/−	−/−	−/−	−/−
ICD22	+/+	−/−	+/+	+/+	−/−	ND	−/−
ICD24	ND	ND	+/+	−/−	−/ND	ND	ND
ICD27	+/+	−/−	+/+	−/−	−/−	−/−	−/−
ICD32	+/+	−/−	+/+	−/−	−/−	−/−	−/−

*+ = Positive; − = negative.
ND = Specimens not obtained or unsatisfactory.

tached to red blood cells) was also carefully noted. Smears from the urethra were considered adequate only if glandular epithelial cells were present. Smears from the cervix were considered adequate only if they contained mucus. Slides from upper genital tract sites were rejected if they were completely devoid of any material. Slides were also rejected if the specimens were so thick it was impossible to discern cellular detail. After examination the smears were frozen to permit later reexamination to determine reproducibility.

Formalin-fixed endometrial tissue was routinely paraffin embedded; 4 μm sections were cut, deparaffinized, and stained as described above with 150 μl of antibody solution. Only specimens containing endometrial glandular epithelium were considered adequate for evaluation by fluorescein-conjugated antibody. All specimens were examined without knowledge of clinical or laboratory findings.

Two years after initial evaluation, all smears from the first 11 women who were found by either culture or fluorescein-conjugated antibody staining to have chlamydial infection were examined, along with an equal number of slides from corresponding sites from the first 16 patients who had negative cultures and negative fluorescein-conjugated antibody staining at all sites. All slides from these 27 patients were relabeled, restained with 150 μl of antibody-containing solution, and incubated for 30 minutes at 37° C. These were then reexamined by the original observer (who had more than 2000 hours of experience) as well as by a second, less experienced observer (who had only 80 hours of prior experience) to assess intraobserver and interobserver reproducibility.

Results

Correlation of culture and serologic results with presence of salpingitis and/or endometritis. On the initial review of 326 smears collected for fluorescein-conjugated antibody staining, 320 were considered adequate for evaluation. Endometrial tissue adequate for fluorescein-conjugated antibody staining was obtained from 50 of 55 patients who underwent endometrial aspiration. Twenty-one (38%) of the patients had positive cultures for *C. trachomatis* at one or more sites; 10 (18%) had positive endometrial or tubal cultures. The remaining 34 had negative cultures for *C. trachomatis* at all sites. Two patients with negative cultures had serologic evidence of acute chlamydial infection: One had serum immunoglobulin M antibody to *C. trachomatis* at the time of enrollment, and the second had a fourfold rise in total antibody titer to *C. trachomatis*. Overall, chlamydial infection was demonstrated by isolation of the agent from any site or by serologic results in 21 (58%) of 36 women with salpingitis and/or plasma endometritis and in two (11%) of 19 with neither salpingitis nor endometritis. None of the 19 women with neither salpingitis nor endometritis had *C. trachomatis* isolated or had positive fluorescein-conjugated antibody staining at the endometrial, tubal, or cul-de-sac sites.

Table II. Correlation of culture and immunofluorescent staining of smears and tissue for detection of *C. trachomatis* by site, in 55 women with suspected pelvic inflammatory disease

Site	*Culture positive, fluorescein-conjugated antibody positive*	*Culture positive, fluorescein-conjugated antibody negative*	*Culture negative, fluorescein-conjugated antibody positive*	*Culture negative, fluorescein-conjugated antibody negative*
Culture-positive patients (n = 21)				
Urethra	9	3	1	5
Rectum	6	2	0	10
Endocervix	15	2	2	2
Endometrium*	7	0	5	9
Fallopian tubes	5	0	5	20
Cul-de-sac	1	0	1	18
Total specimens	43	7	14	64
Culture-negative patients (n = 34)				
Urethra	0	0	0	30
Rectum	0	0	0	30
Endocervix	0	0	0	36
Endometrium	0	0	0	30
Fallopian tubes	0	0	1	33
Cul-de-sac	0	0	0	32
Total specimens	0	0	1	191

*Results of culture and staining of endometrial smears and endometrial biopsy specimens are grouped for each patient.

Correlation of direct immunofluorescent staining with culture results. Fifty-five specimens from 21 patients were culture positive and 58 smears from 22 patients were fluorescein-conjugated antibody positive for *C. trachomatis*. In smears, elementary bodies were seen free, within polymorphonuclear leukocytes and histiocytes, or attached to the surfaces of glandular cells or red blood cells. Intraepithelial inclusions were seen by fluorescein-conjugated antibody in only four of 18 cervical smears (all of which also contained elementary bodies seen by fluorescein-conjugated antibody). Smears from the rectum, urethra, endometrium, fallopian tubes, and cul-de-sac demonstrated only elementary bodies. Examples of cervical smears that do and do not show elementary bodies and inclusions are shown in Fig. 1, *A* and *B*. Larger organisms, which showed peripheral immunofluorescent staining characteristic of chlamydial reticulate bodies, were occasionally seen but only in association with elementary bodies.

Table I compares the results of culture and fluorescein-conjugated antibody stain by site for each of the 22 women with positive cultures and/or fluorescein-conjugated antibody tests at any site. A disagreement between culture and fluorescein-conjugated antibody staining occurred at one site in six patients, at two sites in three patients, and at five sites in two patients. Among the seven patients with discrepancies between cultures and immunofluorescent staining of an upper tract site (positive immunofluorescence with negative culture in all seven), three had a positive culture for *C. trachomatis* at another upper tract site, and three had positive cultures for *C. trachomatis* only from the lower genital tract or rectum, while one had no positive cultures but did have serum immunofluorescent antibody to *C. trachomatis* in a persistently elevated titer of 1:512. All seven had salpingitis.

Table II summarizes the results of all cultures and fluorescein-conjugated antibody stains by site in all 55 patients. The fluorescein-conjugated antibody stain was positive for 43 (86%) of 50 culture-positive specimens, 14 (18%) of 78 culture-negative specimens from patients who had positive cultures at other sites, and one (0.5%) of 192 specimens from patients who had negative cultures at all sites. This latter patient had a serum microimmunofluorescent antibody titer of 1:512 against *C. trachomatis*. Among the 65 specimens that were positive by culture and/or immunofluorescence, those positive by immunofluorescence alone accounted for three of 40 from the lower genital tract or rectum versus 12 of 25 from the endometrium, fallopian tubes, or cul-de-sac ($p \leq 0.001$). Specimens positive by culture only accounted for seven of 40 positive results by either method from the lower tract versus none of 25 results from the upper tract ($p \leq 0.02$). Thus, of 25 upper-tract specimens that were positive by either method, *C. trachomatis* was detected by culture in 13 specimens and by immunofluorescence in 25 specimens.

All lower- and upper-tract fluorescein-conjugated antibody smears that disagreed with culture were then reviewed by three observers. On review of 15 specimens

Table III. Results of culture and direct immunofluorescence for *C. trachomatis* in 320 specimens from 55 patients, stratified on the basis of presence or absence of laparoscopic evidence of salpingitis or histologic evidence of plasma cell endometritis

Site tested by laparoscopic findings	*Culture positive, fluorescein-conjugated antibody positive*	*Culture positive, fluorescein-conjugated antibody negative*	*Culture negative, fluorescein-conjugated antibody positive*	*Culture negative, fluorescein-conjugated antibody negative*
Laparoscopic evidence of salpingitis or endometritis (n = 36 patients)*				
Upper-tract sites	13	0	12	80
Lower-tract sites	28	6	3	57
No laparoscopic evidence of salpingitis or endometritis (n = 19 patients)†				
Upper-tract sites	0	0	0	62
Lower-tract sites	2	1	0	55

*Ten patients had salpingitis only, two had endometritis only, 22 had both salpingitis and endometritis, and two had an endometrial biopsy specimen that was inadequate for histologic examination.

†One patient had an endometrial biopsy specimen that was inadequate for histologic examination.

that were initially interpreted as fluorescein-conjugated antibody positive and culture negative, 10 showed typical elementary bodies, two showed no elementary bodies, and three were considered inadequate after restaining, as discussed below. An unblinded review of the seven negative fluorescein-conjugated antibody smears from culture-positive sites was then performed without restaining. On one urethral smear, three poorly stained elementary bodies were then located. In addition, it was noted that one smear had been stained on the reverse side. The smear was positive after the correct side was stained. One cervical smear failed to show any elementary bodies. All four remaining negative fluorescein-conjugated antibody smears from culture-positive sites had initially been stained with 100 μl of antibody solution for 15 minutes. When these four smears were restained with 150 μl of antibody solution for 30 minutes, all four were found to contain elementary bodies.

Immunofluorescent staining of paraffin-imbedded endometrial biopsies. Fifteen of the 50 paraffin-imbedded endometrial biopsy specimens considered adequate for evaluation were positive by immunofluorescent staining for *C. trachomatis* elementary bodies. Endometrial aspirate cultures were positive for seven of the 11 patients whose endometrial biopsy specimens did show *C. trachomatis* by immunofluorescence versus none of 35 whose biopsy specimens did not show *C. trachomatis* by immunofluorescence. Of the four patients with positive fluorescein-conjugated antibody biopsy specimens who had negative endometrial aspiration cultures, all had positive cultures from other sites, three had characteristic, fluorescein-conjugated antibody–positive chlamydial inclusions within endometrial cells and all had typical elementary bodies of *C. trachomatis* detected by immunofluorescence in endometrial biopsy specimens and smears.

The elementary bodies were located on the surfaces of and within endometrial epithelial cells and in polymorphonuclear leukocytes and histiocytes. In eight of 11 patients whose endometrial biopsy specimens showed elementary bodies, scattered typical inclusions were also found in from one to 70 surface glandular cells, representing fewer than 1% of glandular cells present on the sections reviewed. Elementary bodies were also seen attached to the surface epithelium adjacent to inclusions in all cases where inclusions were seen (Fig. 2, *A* and *B*).

Immunofluorescent staining and culture of fimbrial biopsy specimens and endosalpingeal smears or brushes. Endosalpingeal brushes or smears were available from 28 women for comparison of immunofluorescent staining and culture. Endosalpingeal brushes or smears were positive for *C. trachomatis* (by both fluorescein-conjugated antibody stain and culture) in three cases (P010, P016, and ICD9). An example of an endosalpingeal smear showing elementary bodies of *C. trachomatis* associated with a polymorphonuclear leukocyte is shown in Fig. 3. The fimbrial biopsy specimen was positive (by both fluorescein-conjugated antibody stain and culture) in only one case (P010). One additional patient (P022) underwent salpingo-oophorectomy for tuboovarian abscess; *C. trachomatis* was demonstrated by fluorescein-conjugated antibody stain but not by culture in tubal tissue and aspirated tubal pus from this patient.

Correlation of immunofluorescent staining and culture with laparoscopy. Table III shows the results of culture and direct immunofluorescent stain for *C. trachomatis* stratified by the presence or absence of salpingitis or endometritis. All of the 15 fluorescein-conjugated antibody–positive, culture-negative specimens occurred in patients with laparoscopic evidence of salpingitis. Twelve occurred in upper-tract sites, and three

Table IV. Intraobserver reproducibility in first and second interpretations of immunofluorescent staining by site

Site	*Both positive*	*First positive, second negative*	*First negative, second positive*	*Both negative*
Urethra (n = 19)	5	—	2	12
Rectum (n = 14)	4	—	2	8
Cervix (n = 21)	9	1	1	10
Endometrium (n = 16)	5	2*	1	8
Tubes (n = 16)	5	3†	—	8
Cul-de-sac (n = 12)	1	—	—	11
Total (n = 98)	29	6	6	57

*One of these two specimens was felt to be inadequate on review because of poor counterstaining.
†Two of these three specimens were felt to be inadequate on review because of poor counterstaining.

Table V. Interobserver reproducibility in interpretation of immunofluorescent staining by site

Site	*Both observers: positive*	*Observer A: positive; Observer B: negative*	*Observer A: negative Observer B: positive*	*Both observers: negative*
Urethra (n = 19)	5	2	1	11
Rectum (n = 14)	5	2	0	7
Cervix (n = 20)*	7	2	2	9
Endometrium (n = 16)	6			10†
Tubes (n = 16)	3	2	1	10‡
Cul-de-sac (n = 12)	1	—	—	11
Total (n = 97)	27	8	4	58

Observer A: more experienced; Observer B: less experienced.
*One specimen judged unsatisfactory by Observer B was excluded.
†One specimen was judged inadequate by both observers because of poor counterstaining.
‡Two specimens were judged inadequate by both observers because of poor counterstaining.

in lower-tract sites. All of the seven culture-positive, fluorescein-conjugated antibody–negative specimens were from lower-tract sites, and six occurred in patients with laparoscopic evidence of salpingitis. Overall, among 36 women with pelvic inflammatory disease, upper-tract specimens revealed chlamydia by fluorescein-conjugated antibody stain alone in four women, by culture alone in none, and by both methods in eight women (Table I).

Intraobserver and interobserver reproducibility in interpretation of immunofluorescent staining. Table IV shows the intraobserver reproducibility as assessed by the original observer after slides were restained with 150 ml of antibody-containing solution and incubated for 30 minutes at 37° C. Of 98 specimens that were restained and rescreened, 95 were felt to be satisfactory for review. Fifty-seven smears were called negative on both examinations; 29 were called positive on both examinations; six were called negative, then positive (as described above, all sites were positive by culture and all slides had originally been stained with only 100 ml of antibody, one on the wrong side of the slide, and incubated for only 15 minutes at room temperature); six were initially called positive, then subsequently called negative or inadequate. Of the three that were initially positive, then negative, a final unblinded review disclosed definite elementary bodies in one. Of three that were initially positive, then inadequate, all were from a single patient and showed background fluorescence after restaining. In summary, there was 87% intraobserver reproducibility, despite possible problems attributed to restaining and to initial use of protocol for fluorescein-conjugated antibody staining that may not have been optimal.

Table V shows the results of interobserver reproducibility as assessed on restained slides. Of 97 slides considered adequate for evaluation by both observers, 58 were called negative by both observers, 27 were called positive by both, and there was disagreement on 12 of the specimens. Thus interobserver reproducibility was also 87%. The more experienced observer agreed with culture in 11 of the 12 interobserver disagreements, while the less experienced observer agreed with culture in one of the 12 interobserver disagreements. A final joint review of the cases on which there was disagreement was then performed with the observers blinded as to culture results and their original classification of the slide. In all cases, the final diagnoses were similar to the original diagnosis of the more experienced observer, and in all but one case the final diagnosis was in agreement with culture results at that site.

Comment

In this comparison of conventional culture techniques with direct immunofluorescence with the use of fluorescein-conjugated monoclonal antibodies for detection of *C. trachomatis*, we found that when specimens were regarded as positive if either culture or initial fluorescein-conjugated antibody examination was positive, in lower-tract sites the sensitivity of culture (92%) was higher than that of direct fluorescein-conjugated antibody (83%), whereas for specimens from the endometrium and tubes the sensitivity of direct fluorescein-conjugated antibody (100%) was substantially higher than that of culture (52%). Several factors may be responsible for the lower sensitivity of culture for upper-tract specimens. Infections in upper-tract sites may result in production of specific or nonspecific toxins that interfere with culture. Several studies have shown that whole sera can to some extent neutralize homologous strains of chlamydia.[7, 8] Caldwell and Perry[9] and Peeling et al.[10] noted that antibodies to the major outer membrane protein of *C. trachomatis* (MOMP) could neutralize homologous strains in vitro and that neutralization occurred after endocytosis of the organism, perhaps by promoting phagolysosomal fusion or prevention of intracellular differentiation. Howard[11] showed that neutralization titers of serum antibody were increased by the addition of complement. In the upper tract and especially in the endometrium, where subepithelial hemorrhage is frequently seen adjacent to intracellular chlamydial inclusions, complement, complement-fixing antibody, and perhaps antibody having other functions may be more available than in lower-tract sites and may result in more effective neutralization of organisms. Thus the organisms noted in the upper tract may or may not have been viable at the time of sampling. In addition, surgical procedures, for example, cleaning the cervix with Betadine before endometrial biopsy, could also have interfered with isolation of chlamydia from the upper tract.

The specificity of direct immunofluorescent staining for *C. trachomatis* appears to be high. Fourteen of the 15 culture-negative, fluorescein-conjugated antibody–positive specimens came from patients who had positive cultures at other sites, and the remaining patient had high titers of serum antibody to chlamydia. Among patients who had negative cultures at all sites, the specificity of direct fluorescein-conjugated antibody staining of all specimens was 191 of 192, or 99%. Among the culture-positive specimens, the sensitivity of initial fluorescein-conjugated antibody stain in comparison with culture was 82% for staining with 100 μl of antibody for 15 minutes and 97% for staining with 150 μg for 30 minutes. We therefore have continued to use the latter method. The sensitivity of fluorescein-conjugated antibody staining was lowest for specimens from the urethra and rectum. This may have been related to the concentration of organisms present, to the amount of material collected (collection of adequate material from the rectum and urethra may be more difficult than from other sites), and to the initial use of a staining method that was not optimal in some cases.

Both intraobserver and interobserver reproducibility was 87%. In several cases the intraobserver variability was due to technical problems, such as staining the wrong side of the slide or inadequate counterstaining with the second restaining, or to use of a lower concentration of antibodies with the initial staining, as discussed above. It may also be that repeated washing of the slides during restaining resulted in loss of elementary bodies or that fewer antigenic sites were available for antibody binding after restaining.

Fimbrial biopsy did not increase the detection of *C. trachomatis* in this study, perhaps because of the small amount of tissue examined. All verified tubal chlamydial infections were demonstrated by examination of endosalpingeal brushings or aspirated tubal pus.

The use of monoclonal antibodies on endometrial tissue with identification of the organism within endometrial glandular epithelium, within inflammatory cells, within glandular epithelium, or in underlying endometrial stromal cells allows one to distinguish true endometrial infection from contamination of the endometrial cavity that might result when swabs or curettes are introduced into the uterus through the infected cervix. In the present study, staining of paraffin-embedded endometrial tissue with monoclonal antibody to *C. trachomatis* detected all infections demonstrated by culture and several not demonstrated by culture. Endometrial biopsy also permits confirmation of the presence of endometritis.[8] Thus endometrial biopsy for conventional histologic studies and immunofluorescent staining should be useful in the future for establishing the diagnosis of chlamydial endometritis.

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Obstetric hospital stays by diagnosis related groups: A community-based analysis

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The study analyzed obstetric inpatient hospital stays by diagnosis related group in Sacramento, California, and Syracuse, New York, two areas with similar admission rates, between 1981 and 1984. The sample included 123,308 hospital discharges. The data indicated that aggregate obstetric stays in Syracuse were 32.9% longer than those in Sacramento, which was typical of differences between these stays in the western and northeastern United States. Obstetric stays in both areas declined between 1981 and 1984; however, the rate of decline in Sacramento (6.1%) was substantially higher than that of Syracuse (3.5%). Variability of obstetric stays was substantially lower in Syracuse for most diagnosis related groups, including those with the largest caseloads. These results suggest that community and regional differences in obstetric hospital stays may be produced by system-wide consumer preferences and physician practice patterns, more than by hospital bed availability, health maintenance organization activity, or other factors. (Am J Obstet Gynecol 1986;154:873-8.)

Key words: Obstetrics, delivery, hospitalization, utilization review

The implementation of new hospital reimbursement systems based on diagnosis related groups (DRGs) is focusing increased attention on reduction of acute care stays in the United States. Under these systems, each hospital inpatient is assigned to one of 468 DRGs, which are defined by the individual's principal operating room procedure, principal diagnosis, presence or absence of comorbidities and complications, age, sex, and discharge status.[1] Obstetric patients are assigned to DRGs 370 to 384. Specific categories concern admission for cesarean section (DRGs 370 and 371), vaginal delivery (372 to 375), postpartum diagnoses (376 and 377), ectopic pregnancy (378), threatened abortion (379), abortion (380 and 381), false labor (382), and antepartum diagnoses (383 and 384).[2] Hospitals receive a relatively fixed payment for each discharge in each DRG. A limited additional payment is frequently provided for patients with extremely long stays or inordinately high costs; however, such reimbursement does not approximate the costs of providing acute care.[3]

DRGs were adopted by the federal Health Care Financing Administration for Medicare reimbursement during 1983. Since that time, hospital stays for medicare patients have declined by 6%.[4] Obstetric hospital stays are generally reimbursed through Medicaid, Blue Cross, or commercial insurance. A number of states, including New Jersey, Michigan, Ohio, Pennsylvania, Utah, and Washington, have implemented Medicaid reimbursement systems based on DRGs.[5] Blue Cross plans in various areas of the nation and commercial insurance companies have also implemented DRG-based reimbursement.[6] Connecticut has become one of the first states to employ the federal DRGs for all major payers.[7]

Historically, obstetric hospital stays in the United States have varied substantially by region. Stays have generally been shortest in the western part of the nation and longest in the northeastern and southern regions.

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Table I. Hospital lengths of stay: Obstetric DRGs—total

	Mean annual discharges	*Inpatient mean stays*			
		1981	*1982*	*1983*	*1984*
Sacramento	19,731	2.63	2.50	2.49	2.47
Syracuse	11,096	3.40	3.41	3.29	3.28

In 1977, obstetric mean hospital stays ranged from 2.8 days in the West to 3.9 days in the Northeast, a difference of 39.2%, in comparison with 3.5 days for the entire nation.[8] In 1982, regional mean stays for specific obstetric procedures and diagnoses with the highest frequencies were also shortest in the West and longest in the Northeast. Stays for vaginal delivery without complicating diagnoses ranged from 2.2 days in the West to 3.4 days in the Northeast, a difference of 54.5%, in comparison with a national mean of 2.9 days. Mean stays for cesarean section without substantial complications ranged from 4.8 days in the West to 6.7 days in the Northeast, a difference of 39.6%, in comparison with a national mean of 5.7 days.[9]

Available literature suggests that these and other variations in regional hospital stays may be produced by factors such as the greater availability of hospital beds in the Northeast,[10] the high use of health maintenance organizations in the West,[11] or consumer preferences and physicians' practice patterns.[12] It has also been suggested that the implementation of DRGs by a full range of payers may reduce hospitals stays for the entire nation to levels currently experienced in the West.[13] Obstetricians have also predicted reductions in hospitalization for many of their patients.[14]

However, research has not focused on regional variations in obstetric hospital stays. National studies of major metropolitan areas, such as the work of Knickman and Foltz,[15] have concerned aggregate hospital utilization rather than stays for individual services such as obstetrics. Most of the so-called small-area studies, such as those performed by Wennberg et al.[12, 16] in Maine and Iowa, have evaluated differences in discharges and patient days per thousand, rather than stays, for specific procedures.

This study compared hospital mean stays for obstetric inpatients by DRG category in two U.S. metropolitan areas, Sacramento, California, and Syracuse, New York. These areas are located in the West and Northeast, respectively, the regions that have produced the widest variations in obstetric inpatient stays during recent years. The analysis identified variations and obstetric stays by DRG category and suggested possible causes of these differences.

Method

The study focused on inpatient lengths of stay and related aspects of hospital utilization for obstetric DRGs in Sacramento, California, and Syracuse, New York, during the calendar years 1981 to 1984. These DRGs, which are contained in Major Diagnostic Category (MDC) 14, were defined according to the Yale School of Organization and Management's DRG classification system, first revision.[2]

Sacramento and Syracuse were selected as the areas for the study because they had similar hospital utilization characteristics and resource availability. Total hospital admissions per 1000 population in 1984, 129.56 (Sacramento) and 130.17 (Syracuse), varied by less than 1%. Hospital bed availability per 1000 population in 1984 was also similar: 3.05 (Sacramento) and 2.91 (Syracuse). Percentages of inpatient populations less than 65 years of age differed by 2 points: 78.3 (Sacramento) and 80.1 (Syracuse). Each area also contained a medical school hospital and at least three community hospitals (Hospital Executive Council of Syracuse, unpublished data).

Hospital utilization data for the calendar years 1981, 1982, 1983, and 1984 were collected by the acute care planning organization in the respective areas, the Sacramento-Sierra Hospital Association and the Hospital Executive Council of Syracuse. All 11 Sacramento general hospitals and all four Syracuse hospitals furnished utilization data. This information was processed into DRG data through use of a standard first-revision "Grouper" program, International Classification of Diseases, Adapted, ed. 9, Clinical Modification (ICD-9-CM). A total of 123,308 discharges in the sample metropolitan areas were identified through these activities.

The initial analysis of the data focused on comparison of discharge levels and mean stays for the combined obstetric DRGs in the two areas. Subsequent analyses focused on mean stays and length-of-stay coefficients of variation for each DRG in the two areas. Coefficients of variation (standard deviation divided by mean) were employed to compare variability of stays in order to control for the influence of mean stay levels.[17]

Results

The initial section of the analysis focused on inpatient discharges and mean stays for the combined obstetric DRGs in the two metropolitan areas. Related data are summarized in Table I.

This information indicated that obstetric lengths of stay in Sacramento and Syracuse generally reflected differences between stays in the western and north-

Table II. Hospital lengths of stay: Obstetric DRGs 370 to 377

	Mean annual discharges	*Inpatient mean stays (Stay coefficients of variation)*			
		1981	*1982*	*1983*	*1984*
DRG 370: Cesarean section with complications					
Sacramento	358	7.37	6.24	5.67	5.74
		(0.76)	(0.61)	(0.51)	(0.73)
Syracuse	318	7.61	7.47	6.82	7.40
		(0.81)	(0.62)	(0.54)	(0.63)
DRG 371: Cesarean section without complications					
Sacramento	2,549	4.87	4.68	4.30	4.27
		(0.57)	(0.44)	(0.52)	(0.60)
Syracuse	1,526	6.12	5.97	5.88	5.65
		(0.52)	(0.55)	(0.45)	(0.50)
DRG 372: Vaginal delivery with complicating diagnoses					
Sacramento	709	3.29	2.85	2.77	2.73
		(0.75)	(0.56)	(0.56)	(3.02)
Syracuse	763	3.95	3.76	3.64	3.68
		(0.60)	(0.58)	(0.57)	(0.57)
DRG 373: Vaginal delivery without complicating diagnoses					
Sacramento	11,298	2.29	2.13	2.12	1.97
		(2.26)	(0.83)	(3.34)	(0.43)
Syracuse	5,275	3.07	3.01	2.96	2.83
		(0.41)	(0.52)	(0.54)	(0.45)
DRG 374: Vaginal delivery with Steril./D&C					
Sacramento	655	3.01	2.84	2.73	2.74
		(0.48)	(0.58)	(0.35)	(0.61)
Syracuse	293	3.57	3.49	3.47	3.58
		(0.33)	(0.27)	(0.25)	(0.32)
DRG 375: Vaginal delivery with OR ex. Steril./D&C					
Sacramento	7	3.00	8.00	2.70	6.80
		(0.73)	(0.43)	(0.72)	(1.27)
Syracuse	4	5.20	2.50	4.50	6.29
		(0.42)	(0.25)	(0.16)	(0.43)
DRG 376: Postpartum diagnosis without OR procedure					
Sacramento	193	3.12	3.11	2.55	2.63
		(0.75)	(0.87)	(1.02)	(0.93)
Syracuse	60	4.25	3.66	2.94	3.85
		(0.66)	(0.68)	(0.76)	(0.71)
DRG 377: Postpartum diagnosis with OR procedures					
Sacramento	87	2.81	2.81	2.18	2.86
		(1.68)	(1.08)	(0.95)	(1.12)
Syracuse	36	3.38	2.63	2.66	3.18
		(1.17)	(1.10)	(1.02)	(1.09)

Steril./D&C = sterilization/dilation and curettage; OR = operating room; ex. = except.

eastern United States that have been identified in the literature.[8, 9] Most of the differences in hospital utilization between the two metropolitan areas could be attributed to variations in lengths of stay rather than admissions. Mean annual obstetric hospital admissions and discharges per 1000 population ranged from 19.57 in Sacramento to 19.97 in Syracuse during the 4-year period, a difference of only 2.0%. At the same time, the mean inpatient stay for Syracuse (3.35 days) exceeded the mean for Sacramento (2.52 days) by 32.9%.

The data also indicated that mean obstetric stays declined in both areas between 1981 and 1984. With the exception of the years 1981 and 1982 in Syracuse, a steady progression of year-to-year reductions in stays occurred in both areas. Stays in Sacramento dropped by 6.1%, whereas stays in Syracuse fell by 3.5%. It should be noted that the rate of decline in Sacramento, the area with shorter stays through the period, was almost double that of Syracuse.

The remainder of the study focused on analysis of differences between obstetric hospital stays in the two areas by DRG category. Data concerning DRGs 370 to 377, which concern deliveries and postpartum diagnoses, are summarized in Table II.

Table III. Hospital lengths of stay: Obstetric diagnosis related groups 378 to 384

	Mean annual discharges	*Inpatient mean stays (stay coefficients of variation)*			
		1981	*1982*	*1983*	*1984*
DRG 378: Ectopic pregnancy					
Sacramento	334	4.09	4.06	3.75	3.75
		(0.30)	(0.54)	(0.29)	(0.30)
Syracuse	124	5.45	5.40	5.07	4.92
		(0.41)	(0.37)	(0.33)	(0.31)
DRG 379: Threatened abortion					
Sacramento	297	3.02	2.98	2.38	2.32
		(1.22)	(1.73)	(0.89)	(1.32)
Syracuse	216	2.74	2.69	2.54	2.95
		(1.02)	(1.00)	(1.06)	(1.10)
DRG 380: Abortion without D&C					
Sacramento	600	1.18	1.15	1.13	1.09
		(1.14)	(0.67)	(0.51)	(0.50)
Syracuse	284	1.65	1.94	1.60	1.78
		(0.51)	(0.76)	(0.61)	(0.70)
DRG 381: Abortion with D&C					
Sacramento	1523	1.11	1.13	1.20	1.11
		(0.56)	(0.89)	(4.13)	(0.70)
Syracuse	563	1.51	1.46	1.37	1.26
		(0.89)	(0.88)	(0.71)	(0.70)
DRG 382: False labor					
Sacramento	313	2.07	1.18	1.12	1.17
		(9.08)	(0.65)	(0.43)	(0.61)
Syracuse	504	1.08	1.02	1.08	1.08
		(0.57)	(0.56)	(0.31)	(0.48)
DRG 383: Other antepartum diagnoses with complications					
Sacramento	476	3.03	3.07	3.02	2.84
		(0.75)	(0.89)	(0.86)	(0.80)
Syracuse	725	2.59	2.69	2.50	2.47
		(0.89)	(0.89)	(1.28)	(0.90)
DRG 384: Other antepartum diagnoses without complications					
Sacramento	332	2.54	2.33	2.14	1.89
		(1.62)	(1.40)	(1.41)	(1.40)
Syracuse	405	2.33	2.73	2.30	2.47
		(0.94)	(1.50)	(1.26)	(1.22)

D&C = Dilation and curettage.

These data indicated that obstetric DRGs, which included delivery (Nos. 370 to 375), accounted for a substantial majority of all obstetric admissions in both areas, 78.9% of the total in Sacramento and 73.7% of the total in Syracuse. Among obstetric DRGs involving delivery, mean stays for Syracuse, the northeastern community, generally exceeded those of Sacramento. Differences between hospital stays in DRGs with the largest caseloads were substantial. For normal vaginal delivery (DRG 373) the combined average stay for 1981 to 1984 in Syracuse exceeded that of Sacramento by 39.4%, whereas the combined average for cesarean section without comorbidities or complications (DRG 371) in Syracuse exceeded that of Sacramento by 30.5%. Similar differences in stays characterized postpartum diagnoses. These differences were typical of regional variations in stays.

The data also indicated that mean hospital stays for delivery and postpartum DRGs progressively declined in both areas between 1981 and 1984. Reflecting trends in total obstetric stays, however, stays in Sacramento fell more rapidly than those in Syracuse. Rates of decline for DRG 371 and DRG 373 stays in Sacramento, about 16%, were approximately double those of Syracuse.

Perhaps most interesting were the relationships between mean stay levels and variability of hospital stays, as measured by coefficients of variation, which appeared in the data. This information indicated that length of stay variability in Syracuse was comparable to or less than that in Sacramento for most of the DRGs evaluated. Between 1981 and 1984, coefficients of variation for Syracuse averaged lower than those for Sacramento in all categories involving delivery except DRG 370, where they were essentially equal. Variability of stays was also lower in Syracuse for postpartum diagnoses. Coefficients of variation for postpartum diagnoses with operating room procedures (DRG 377) were substantially higher in both areas than coefficients for other DRGs, indicating that this category included patients with an extensive range of hospital stays.

The second portion of the DRG-specific section of the study focused on categories involving problem pregnancies, other antepartum diagnoses, and inpatient abortions. Related data are summarized in Table III.

These combined categories accounted for 20% to 25% of total obstetric admissions in the two metropolitan areas. Utilization varied between the two areas. Admission levels were highest for abortions with curettage (DRG 381) in Sacramento and for other antepartum diagnoses with complications (DRG 383) in Syracuse. Mean stays in Syracuse generally exceeded those in Sacramento for DRGs involving ectopic pregnancy, abortions, and antepartum diagnoses without complications. However, mean stays in Sacramento generally exceeded those in Syracuse for DRGs involving false labor and antepartum diagnoses with complications. The data did not suggest any relationships between these two groups of DRGs, especially with regard to antepartum diagnoses.

The data indicated that hospital stays for a number of these DRGs declined between 1981 and 1984, although such reductions were not as prevalent as among DRGs involving delivery and postpartum diagnoses. Over the period of the study, mean stays declined in Sacramento but increased or remained stable in Syracuse in DRGs 379, 380, 382, and 384.

These trends in hospital mean stays did not appear to be related to data concerning variability of stays. For the 4-year period, average coefficients of variation were lower in Syracuse for all of these DRGs except No. 378, where they were essentially equal, and for No. 383, where Sacramento coefficients were lower. Coefficients of variation for both areas were especially high in DRG 384, indicating that this category included patients with an extensive range of hospital stays.

Comment

As DRG-based hospital payment systems are implemented for Medicaid, Blue Cross, commercial insurance, and other private payers, it seems that probable increased attention will focus on reduction of obstetric inpatient stays. Obstetric cases constitute a major portion of the claims volume among these payers. Although wholesale elimination of obstetric stays through extensive use of birthing centers may remain a long-term goal, short-term utilization initiatives will probably concentrate on length-of-stay reduction, particularly in the northeastern United States.

This comparison of obstetric stays by DRG focused on two metropolitan areas with acute care bed availability substantially below national levels. As hospital bed reduction proceeds in the rest of the nation, the experience of Sacramento and Syracuse areas may anticipate national trends. The analysis identified substantial differences in obstetric hospital mean stays between these two areas. These differences reflected the longer hospital stays that have marked obstetric utilization in the northeastern United States. Utilization data indicated that although stays declined in both areas between 1981 and 1984, the rate of decline in Sacramento was almost double that of Syracuse. Syracuse stays fell at a lower rate in DRGs involving delivery and postpartum diagnoses and remained stable or increased in other categories. If these trends are representative of regional patterns, it appears that the gap between obstetric stays in western and northeastern United States may be widening.

The analysis also provided some information concerning the causes of these differences in stays. The data indicated that acute care bed availability and admission rates were not responsible for length-of-stay variations, since these levels were essentially the same in Sacramento and Syracuse. HMO penetration in Sacramento was substantially higher than in Syracuse. However, the data also suggested that greater uniformity of physician practice, typically generated by extensive use of health maintenance organizations and other utilization control mechanisms, was probably not a major factor, since length-of-stay variability in Syracuse was lower than that of Sacramento for a substantial majority of the DRGs evaluated. Instead, the differences in mean stays appeared to have been produced by more conservative consumer preferences and physician practice patterns in the Northeast. It was notable that in most obstetric DRGs, especially the high-volume delivery categories, these discharge patterns were less variable than the approaches that produced shorter stays in Sacramento. It is possible that these trends limited the rate of obstetric stay reduction in Syracuse and the Northeast.

These results indicate that regional differences in obstetric stays may be less subject to change than was previously thought. Unlike hospital bed availability and health maintenance organization penetration, which have evolved fairly rapidly in recent years, the behavior of consumer and physician communities will be more difficult to change. The extension of DRG-based hospital reimbursement may help bring this about. However, national uniformity of obstetric hospital stays may not be as close as some would believe.

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An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody

Platelet count and antiplatelet antibody in 116 consecutive pregnancies: Relationship to neonatal platelet count

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Twenty-eight (24%) of 116 pregnant women studied prospectively during an 8-month period in 1983 had platelet counts of <150,000/mm³ at least once during pregnancy. Thirteen of these were thrombocytopenic in both the prenatal and the peripartum period. Eighteen were restudied 3 to 12 months after delivery. One woman, who was pregnant again, had a platelet count of 140,000/mm³. In the others, platelet counts were in the normal range. Platelet-associated immunoglobulin G and serum antiplatelet antibody levels were elevated in 79% and 61%, respectively, of these 28 women on at least one occasion. However, 59% of 73 pregnant nonthrombocytopenic women had increased platelet-associated immunoglobulin G levels and 59% had positive serum antiplatelet antibody test results. Twenty women who had increased platelet-associated immunoglobulin G levels and positive serum antiplatelet antibody test results were normal 6 to 10 months after delivery. Of 105 infants studied, 10 were thrombocytopenic. Neonatal thrombocytopenia was not predicted by maternal platelet count, platelet-associated immunoglobulin G, or serum antiplatelet antibody. By the fall of 1984, the incidence of thrombocytopenia had dropped to two in 280 consecutive pregnancies. We conclude that (1) epidemics of thrombocytopenia can occur in pregnant women and (2) if a women is found to be thrombocytopenic for the first time during pregnancy, she should not be subjected to the measures advocated for the management of pregnancy in women with autoimmune thrombocytopenic purpura. (Am J Obstet Gynecol 1986;154:878-82.)

Key words: Thrombocytopenia, platelets, pregnancy, antiplatelet antibody

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During the last 6 months of 1982, a surprisingly large number of asymptomatic women receiving antenatal care at New York University Medical Center were reported to have low platelet counts. At this time the Medical Center introduced new cell-counting machines into the clinical hematology laboratories. These machines provide an unsolicited platelet count on every blood sample submitted. Thus for the first time platelet counts were being reported in asymptomatic pregnant women.

To determine the incidence of thrombocytopenia, we conducted a prospective study of 116 consecutive pregnancies from February, 1983, to September, 1983. We measured platelet-associated immunoglobulin G and tested for the presence of serum antiplatelet antibody in these women. We also obtained platelet counts on their infants to determine if these maternal factors affected the values of the infants.

Methods

The prospective study was conducted from February, 1983, through September, 1983. One hundred sixteen consecutive unselected pregnant women gave informed consent and enrolled in this study during routine prenatal care visits at Bellevue Hospital. Clinical data recorded for all women included age, parity, last menstrual period, gestational age by ultrasound, blood type, and prior medical problems. Complications of pregnancy, labor, delivery, and postpartum course were noted. Platelet counts and antiplatelet antibody studies were performed during at least one prenatal care visit, at the time of admission for delivery, and during the first week post partum. For the purposes of this study, "prenatal" refers to data acquired during a prenatal outpatient visit, up to week 39 of gestation. "Peripartum" refers to data collected from 3 days before delivery through 7 days post partum. Cord serum was collected from 58 deliveries. Birth weight, blood type, gestational age by Dubowitz scoring,[1] and neonatal complications were noted for all infants. Platelet counts were performed on 105 newborn infants.

Blood anticoagulated with ethylenediaminetetraacetic acid was collected for measurement of platelet count and platelet-associated immunoglobulin G. Serum for determination of serum antiplatelet antibody was stored frozen at −20° C until assay, within 1 week. Platelet counts were performed on the day of collection either by an automated cell counter (Coulter Model S-Plus IV, Hialeah, Florida, Ortho Model ELT 800, Raritan, New Jersey) or by the manual technique of Brecker and Cronkite.[2] Low platelet counts were confirmed by review of the peripheral blood smear. Platelet-associated immunoglobulin G and serum antiplatelet antibody were quantitated by solid phase radioimmunoassay as described by Faig and Karpatkin.[3]

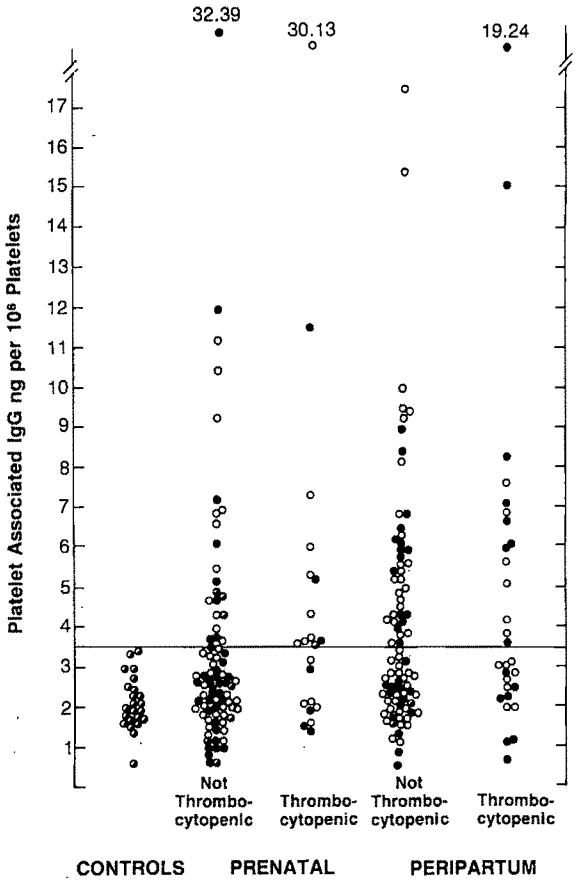


Fig. 1. The level of platelet-associated immunoglobulin G is shown for 23 healthy nonpregnant women, for 28 women who were thrombocytopenic during pregnancy, and for 73 women with uncomplicated pregnancies (not thrombocytopenic, not toxemic, normal infant). Prenatal and peripartum values are given. *Solid circles* indicate that serum antiplatelet antibody results were positive; *open circles* indicate that serum antiplatelet antibody results were negative; *half-filled circles* indicate that serum antiplatelet antibody was not tested. In some women more than one determination was made and results are shown for each determination. Fifty-one percent of determinations in thrombocytopenic women showed increased platelet-associated immunoglobulin G levels and 44% had positive serum antiplatelet antibody results. Seventy-nine percent of thrombocytopenic women had increased platelet-associated immunoglobulin G levels and 61% had positive serum antiplatelet antibody results on at least one occasion. In the nonthrombocytopenic group 36% of platelet-associated immunoglobulin G determinations were positive and 41% had positive serum antiplatelet antibody results. Fifty-nine percent of these women had elevated platelet-associated immunoglobulin G levels on at least one occasion and 59% had at least one positive serum antiplatelet antibody result.

Briefly, in the platelet-associated immunoglobulin G assay, known numbers of washed platelets of a patient are applied to plastic microtiter plates. Adherent platelets are incubated with rabbit antihuman immunoglobulin G. Bound rabbit antiserum is recognized by iodine 125–labeled staphylococcal protein A. Platelet-associ-

Table I. Clinical data on 10 thrombocytopenic infants

Case no.	Minimum platelet count	Day	Complications	Cord serum antiplatelet antibody	Maternal			
					Minimum platelet count	Maximum platelet-associated immunoglobulin G	Serum antiplatelet antibody	Toxemia
1	44	2	Small for gestational age	ND	158	2.56	+	
2	83	0	None	ND	106	3.0	−	
3	92	5	Respiratory distress Sepsis	ND	141	2.47	+	+
4	100	4	Prematurity Birth asphyxia	ND	180	2.22	−	
5	109	0	Fever Sepsis	ND	160	1.88	+	+
6	110	1	Meconium aspiration	Neg.	246	7.39	−	
7	120	0	Respiratory distress Congenital anomalies	ND	255	4.21	+	
8	132	2	None	Neg.	295	10.97	+	
9	148	5	Premature rupture of membranes Maternal drug abuse	Neg.	67	23.87	+	
10	149	2	None	Neg.	225	3.07	+	

ND = Not determined.

ated immunoglobulin G is calculated with reference to pure human immunoglobulin G (Miles Laboratories, Elkhart, Indiana) added directly to the microtiter plates. Samples from 23 healthy nonpregnant women of childbearing age were assayed during the same time period and served as controls for this test. Platelet-associated immunoglobulin G was considered to be positive if the level was >3.46 ng/10^6 platelets, which is the control mean (2.10 ng/10^6 platelets) plus two times the SD (0.68) for nonpregnant women of childbearing age.

In the assay for serum antiplatelet antibody, normal washed platelets are applied to the plastic microtiter plate. Titered patient serum is added to adherent platelets. Bound immunoglobulin G is detected by incubation with ^{125}I-labeled staphylococcal protein A. Serum antiplatelet antibody is considered to be positive if the amount in counts per minute exceeds 1.3 times the counts per minute of pooled control serum in the same assay (>2 SD above the mean).[3]

Statistical analyses were done with Student's *t* test and regression analysis.

Results

In a prospective evaluation of 116 pregnant women, 28 (24%) were noted to be thrombocytopenic at some time during the course of the study. None of these women had a history of autoimmune thrombocytopenic purpura, although one woman had inactive systemic lupus erythematosus not requiring treatment. Nineteen women were thrombocytopenic at a prenatal visit (platelet count 61,000 to 149,000/mm^3) and 13 of these were still thrombocytopenic in the peripartum period. Nine additional women had become thrombocytopenic by the peripartum period (platelet count 43,000 to 149,000/mm^3).

Eighteen of the 28 thrombocytopenic women returned for follow-up platelet counts 3 months to 1 year post partum. One woman, who was pregnant again, had a platelet count of 140,000/mm^3. The other women all had normal platelet counts at follow-up (range 153,000 to 435,000/mm^3).

Fig. 1 shows the results of antiplatelet antibody studies in 23 nonpregnant women of childbearing age, in the 28 women who were thrombocytopenic, and in 73 women with normal platelet counts and uncomplicated pregnancies. Each point represents the level of platelet-associated immunoglobulin G as shown on the y axis. *Open circles* indicate that test results for serum antiplatelet antibody were negative; *closed circles,* that results were positive; and *half-filled circles,* that serum antiplatelet antibody was not measured. Some of the pregnant women were tested on more than one occasion. Seventy-nine percent of these thrombocytopenic women had increased platelet-associated immunoglobulin G levels and 61% had positive results for serum antiplatelet antibody on at least one occasion. There was no correlation between increased platelet-associated immunoglobulin G levels and positive results of serum antiplatelet antibody. Of the nonthrombocytopenic women, 59% had elevated platelet-associated immunoglobulin G levels on at least on occasion, and 59% had at least one positive test result for serum antiplatelet antibody.

The mean values and range were not different from those in the thrombocytopenic women. As in the thrombocytopenic women, the platelet-associated immunoglobulin G level did not correlate with the presence of serum antiplatelet antibody. Postpartum levels of platelet-associated immunoglobulin G were significantly higher than third-trimester values (4.04 ± 0.41 versus 2.94 ± 0.23 ng/10^6 platelets, mean ± SEM, 60 pairs, $p < 0.02$). Parity had no effect on antiplatelet antibody levels, nor did a blood group incompatibility between mother and fetus.

Fifteen women became toxemic during pregnancy. Six were thrombocytopenic. The proportions of elevated platelet-associated immunoglobulin G levels and positive serum antiplatelet antibody results were similar to those observed in thrombocytopenic women without toxemia as well as in women with normal platelet counts.

Twenty women who had increased platelet-associated immunoglobulin G levels (12) and/or positive serum antiplatelet antibody results (nine) were studied 6 to 10 months after delivery. Thirteen had been thrombocytopenic during pregnancy. All had platelet-associated immunoglobulin G levels of <3.46 ng/10^6 platelets, negative serum antiplatelet antibody results, and normal platelet counts.

Platelet counts were done for 105 neonates whose mothers were enrolled in the study. Ten infants became thrombocytopenic by 5 days of age. Clinical and laboratory data for these infants are shown in Table I. Six thrombocytopenic infants had neonatal complications that could be associated with thrombocytopenia; however, these infants were included since it was not known if immune factors played a role in causing thrombocytopenia. Maternal platlet count, platelet-associated immunoglobulin G levels, and serum antiplatelet antibody results were not predictive of neonatal thrombocytopenia (Table II). Among 68 women who had elevated platelet-associated immunoglobulin G levels on at least one occasion, only four gave birth to a thrombocytopenic infant. Of 37 women in whom platelet-associated immunoglobulin G levels were never elevated, six gave birth to a thrombocytopenic infant. Of 64 women with positive serum antiplatelet antibody results, seven had thrombocytopenic offspring. Three of 41 women with consistently negative serum antiplatelet antibody results had thrombocytopenic infants.

Cord serum antiplatelet antibody results were positive in only four of 58 samples tested (6.9%). These four infants were not thrombocytopenic (platelet count 150,000 to 334,000/mm³), although one of the four mothers was thrombocytopenic, with a platelet count of 95,000/mm³.

After the study had been completed and while the article was in preparation it was noted that the hematology staff were being consulted less frequently about pregnant women with low platelet counts. Therefore the routine laboratory data on 200 consecutive women who attended the prenatal clinic during the fall of 1984 were examined. Only two had a platelet count <150,000/mm³.

Table II. The incidence of neonatal thrombocytopenia among 105 infants with regard to maternal factors

Maternal factor	*No.*	*Thrombocytopenic infants*	
		n	%
Thrombocytopenic	26	3	12
Nonthrombocytopenic	79	7	9
Positive platelet-associated immunoglobulin G	68	4	6
Negative platelet-associated immunoglobulin G	37	6	16
Positive serum antiplatelet antibody	64	7	11
Negative serum antiplatelet antibody	41	3	7

Comment

Twenty-eight of 116 healthy pregnant women seen at this Medical Center in an 8-month period in 1983 had a low platelet count at least once during pregnancy. Published studies of platelet counts during normal pregnancy do not report such a high incidence of thrombocytopenia, and this is also true of our own more recent experience. However, review of the literature does reveal that platelet counts below the normal range do occur in uncomplicatd pregnancy. In 1948 Ward and MacArthur[4] performed platelet counts for 200 healthy women each month from the second month of pregnancy until term. They published the minimum and maximum counts for each month. In every month there were counts of <150,000/mm³ and the minimum counts ranged from 54,000/mm³ (eighth month) to 85,000/mm³ (fifth month).[4] In 1956, Fresh et al.[5] performed platelet counts at the time of delivery in 32 normal pregnancies and reported counts as low as 105,000/mm³. Bonnar and McNicol,[6] in 1969, carried out multiple counts for 10 women between 12 weeks of pregnancy and term and recorded seven counts of <150,000/mm³. Sejeny et al.[7] studied 405 normal women during pregnancy. They reported a significant progressive fall in platelet count during the course of the pregnancy and commented that the lowest counts in some women were outside the normal range. The lowest count recorded was 75,000/mm³.

In 1982, Kelton et al.[8] published a study of 41 pregnancies in 38 patients clinically diagnosed as having immune thrombocytopenia. Out of 23 patients avail-

able for follow-up, the platelet count returned to normal in 11 without therapy after delivery. One may speculate that these 11 patients did not have classic autoimmune thrombocytopenia, but as in our patients the thrombocytopenia was a pregnancy-related phenomenon. Only one half of the thrombocytopenic mothers studied by Kelton et al. had elevated platelet-associated immunoglobulin G levels, similar to our finding of 59%. This is in contrast to the usual finding in patients with autoimmune thrombocytopenia where approximately 90% have elevated platelet-associated immunoglobulin G levels.[3]

It is of interest that the incidence of low platelet counts fell dramatically within a year of completion of the study. One possible explanation for this apparently limited "epidemic" of thrombocytopenia in pregnancy is that the counts were not really low but were an artifact produced by the cell counters. This explanation can be discounted because each time a low platelet count was recorded, a stained blood smear was examined by an experienced observer in order to confirm the accuracy of the count. In addition, of the 19 women who were found to be thrombocytopenic in the prenatal period, 13 were still thrombocytopenic perinatally, making the possibility of a machine error extremely unlikely.

A possible explanation is that the thrombocytopenia was a response to a virus that was prevalent in the community during the period of the study and subsequently disappeared. Viral infections are known to cause transient thrombocytopenia, frequently accompanied by the deposition of immune complexes on platelets.[9] The question that then arises is why was this phenomenon observed only in pregnant women? Possible explanations are: (1) that the pregnant state made the women more susceptible to the hypothetical virus, (2) that platelets become altered during the pregnant state, causing immune complexes to bind to them more readily, or (3) that thrombocytopenia was in fact occurring in healthy people who were not pregnant but this occurrence was not noticed because platelet counts were not performed for these people. This latter explanation is unlikely because a platelet count is obtained for healthy people entering the hospital for routine surgical procedures and no such epidemic of thrombocytopenia was noted among this group of patients during the time this study was carried out. Masson et al.[10] reported immune complexes in the sera of all of 55 healthy pregnant women studied, although others[11,12] have been unable to confirm this. It is possible that the combination of a viral infection and the pregnant state resulted in formation of immune complexes, which in some women bound to the platelets.

Not all women with immunoglobulin G demonstrable on the platelets were thrombocytopenic. This is not surprising because recent studies in nonthrombocytopenic homosexual men and intravenous drug abusers at risk for acquired immune deficiency syndrome, who have been exposed to human T-lymphotropic virus type III, show that they have immunoglobulin G–containing circulating immune complexes with markedly elevated immunoglobulin G on the platelets, suggesting the deposition of immune complexes.[13,14] The incidence of thrombocytopenia in these patients may reflect other influences such as activity of the reticuloendothelial system and the ability of the bone marrow to compensate for shortened platelet life span.

One reason for undertaking this study was to identify any relationship between maternal platelet count, platelet-associated immunoglobulin G, and serum antiplatelet antibody and the platelet count in the neonate. No relationship was demonstrated. Ten infants were thrombocytopenic in the neonatal period (Table I) but maternal platelet count, platelet-associated immunoglobulin G, and serum antiplatelet antibody were not predictive of thrombocytopenia. Indeed six of these infants had other reasons for thrombocytopenia, including sepsis.

As a result of this study we suggest that if a woman is found to be thrombocytopenic for the first time during pregnancy it should not automatically be assumed that she had autoimmune thrombocytopenic purpura and she should not be subjected to the measures usually advocated for management of pregnancy in a woman with autoimmune thrombocytopenic purpura.[15-17] Indeed one woman had a platelet count of 49,000/mm^3, but her child was not thrombocytopenic. Platelet antibody studies will not be helpful in identifying women who have autoimmune thrombocytopenia. If the index of suspicion is high for autoimmune disease (history of long-term bleeding tendency, history of other autoimmune disease), these women may be treated as for autoimmune thrombocytopenic purpura, and their pregnancies managed accordingly. If the diagnosis of autoimmune thrombocytopenic purpura cannot be made, it would seem that the infants may be safely delivered vaginally without the necessity for maternal steroid therapy or fetal scalp platelet count.

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Longitudinal study of platelet indices during normal pregnancy

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The purpose of this longitudinal, prospective study was to define platelet indices during normal pregnancy and to compare them to normal nonpregnant values. Indices evaluated included platelet count, mean platelet volume, and platelet distribution width. No significant change occurred in the mean platelet count or mean platelet volume from the second to the third trimester; however, platelet distribution width increased progressively and significantly during this interval ($p < 0.0001$). Mean platelet volume versus platelet count showed a significant inverse relationship ($p < 0.0001$) and was congruent with normal nonpregnant values. Mean platelet volume versus platelet distribution width exhibited a significant direct relationship ($p < 0.03$) that differed remarkably from normal nonpregnant values. These data support the concept of normal pregnancy as a compensated state of progressive platelet consumption. These findings may have important diagnostic and prognostic applications in discerning acute states of platelet consumption superimposed on the compensated consumption of normal pregnancy. (AM J OBSTET GYNECOL 1986;154:883-7.)

Key words: Platelet count, mean platelet volume, platelet distribution width, thrombocytopoiesis, preeclampsia

Prior to the introduction of automated hematology analyzers, 11 studies of platelet count during normal pregnancy reported either an increase, a decrease, or no change with gestation.[1, 2] Since the advent of automated instruments, there have been eight reports of platelet count during pregnancy.[3-7] Three of these studies were longitudinal in design and reported no significant difference in the mean platelet count between the second and third trimesters.[5-7]

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Additionally, automation facilitated the evaluation of mean platelet volume and platelet distribution width in health and disease.[8, 9] These indices have been shown to reflect bone marrow thrombocytopoietic activity that precedes changes in the platelet count. Fay et al.[4] have recently investigated platelet count, mean platelet volume, and platelet distribution width in a cross-sectional study and interpreted their data to suggest platelet "hyperdestruction" throughout normal pregnancy. In our study, these platelet indices are evaluated longitudinally in uncomplicated pregnancy.

Material and methods

The study population consisted of 51 pregnant women ranging in age from 15 to 32 years who at-

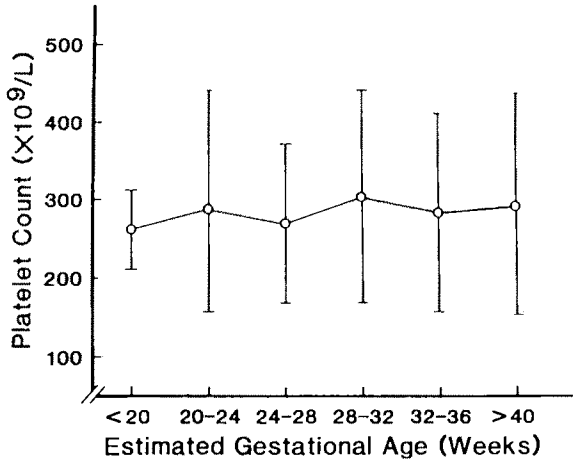


Fig. 1. Platelet count in normal pregnancy (mean ± 2 SD).

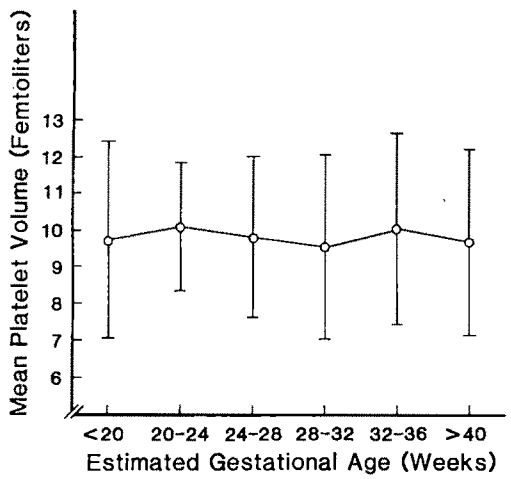


Fig. 2. Mean platelet volume in normal pregnancy (mean ± 2SD).

tended the antenatal clinics of the University of South Alabama Medical Center. Racial distribution was 60% black and 40% white. Blood samples were obtained from those routinely collected for the purpose of quantifying hemoglobin; no additional blood was required for our study. An aliquot of blood obtained by antecubital venipuncture into Vacutainer blood-collecting tubes containing disodium ethylenediaminetetra-acetic acid was analyzed 2 to 24 hours following collection with the use of a Coulter Counter Model S Plus II (Coulter Electronics, Inc., Hialeah, Florida) that was subjected to daily quality control. Platelet count, mean platelet volume, and platelet distribution width were generated synchronously with complete blood count. The mean platelet volume, measured in femtoliters, is defined as the aggregate volume of platelets divided by the platelet count; platelet distribution width is an index of the range of the volume distribution of the platelet population mathematically extrapolated for best fit.[8]

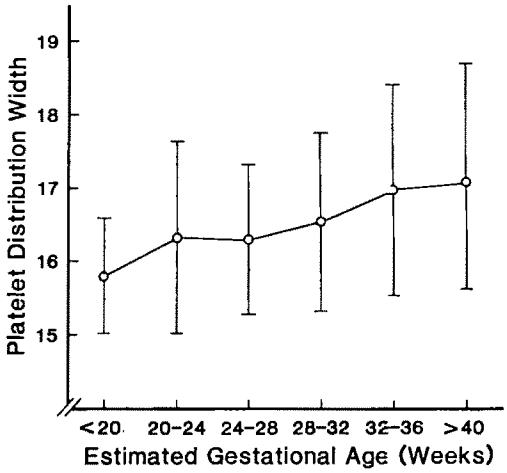


Fig. 3. Platelet distribution width in normal pregnancy (mean ± 2 SD). Groups differ significantly with gestation ($p < 0.0001$).

The platelet distribution width has not been released for clinical use.

Specimens were collected serially, coincident with clinic visits, between 20 and 43 weeks' estimated gestational age. The estimated gestational age was estimated clinically and/or ultrasonographically. All patients received oral iron supplements (ferrous sulfate, 65 mg daily) and maintained a hemoglobin level above 10 gm/dl. Pregnancies complicated by hypertensive disorders were excluded. Samples obtained from laboring patients were also excluded from this analysis.

The following relationships were statistically analyzed as the mean ± 2 SD (platelet count vs. estimated gestational age, mean platelet volume vs. estimated gestational age, platelet distribution width vs. estimated gestational age) or by the linear regression model for best least squares fit ±2 SD of the overall mean (mean platelet volume vs. platelet count, mean platelet volume vs. platelet distribution width). The level of statistical significance was set at 0.05. For descriptive and comparative purposes, data presented in Figs. 1 to 3 are grouped into 4-week intervals and the mean ± 2 SD of these intervals is graphically displayed. Nomograms were constructed and compared with published ranges for normal nonpregnant individuals generated on comparable instruments with the use of the manufacturer's controls.[10, 11]

Results

One hundred ninety-two samples were collected from 51 patients. From this total, 31 observations were excluded from the group analysis. Thirteen samples were obtained from patients who were in labor or who developed preeclampsia. An additional 18 samples were excluded because they did not represent longi-

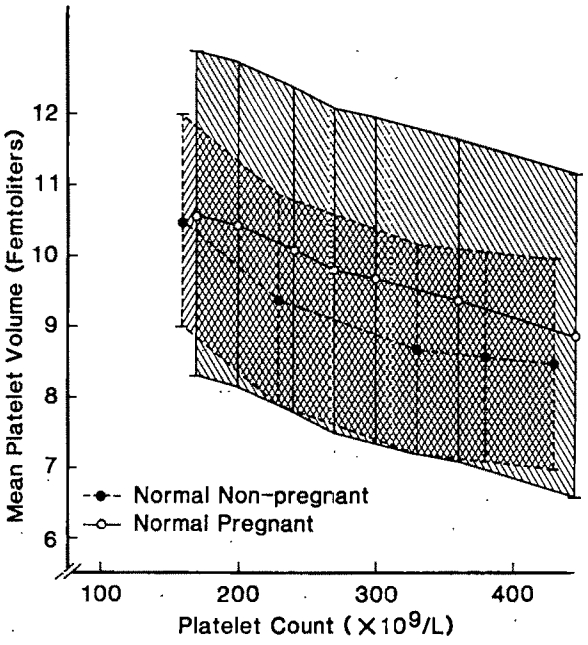


Fig. 4. Mean platelet volume versus platelet count in normal pregnancy *(solid line)* (best least squares model ±2 SD overall mean) exhibiting a significant inverse relationship ($p < 0.0001$). Mean platelet volume versus platelet count in normal nonpregnant population *(dashed line)* (mean ± 2 SD). (Used with permission from Bessman JD, Williams LJ, Gilmer PR. The inverse relationship of platelet size and count in normal subjects, and an artifact of other particles. Am J Clin Pathol 1981;76:289.)

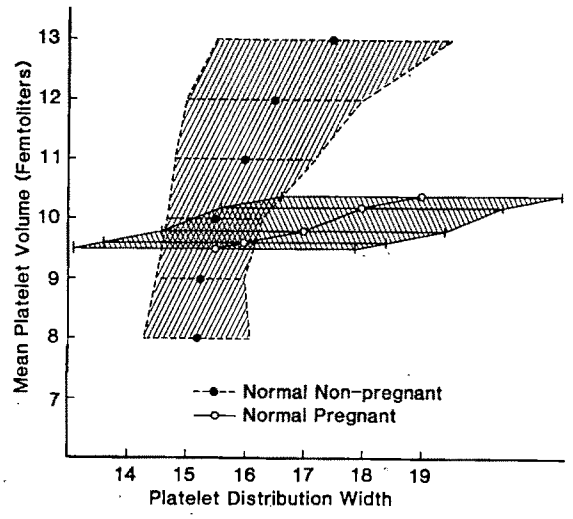


Fig. 5. Mean platelet volume versus platelet distribution width in normal pregnancy *(solid line)* (best least squares model ±2 SD of overall mean) exhibiting a significant direct relationship ($p < 0.03$). Mean platelet volume versus platelet distribution width in normal nonpregnant population *(dashed line)* (mean ± 2 SD). (Used with permission from Bessman JD, Williams LJ, Gilmer PR. Platelet size in health and hematologic disease. Am J Clin Pathol 1982;78:150.)

tudinal observations. The remaining 161 samples from 41 patients were analyzed.

Fig. 1 illustrates the relationship of platelet count versus estimated gestational age. Fig. 2 demonstrates mean platelet volume versus estimated gestational age. Significant change across the second and third trimesters could not be demonstrated. The mean platelet volume ranged from 7.0 to 13.1 fl.

Fig. 3 illustrates platelet distribution width versus estimated gestational age. Platelet distribution width increased progressively with advancing estimated gestational age ($r = 0.53$; $p < 0.0001$). Platelet distribution width ranged from 15 to 19.

Fig. 4 illustrates the mean platelet volume versus platelet count and compares this range with the reference range for a normal nonpregnant population (data expressed as mean ± 2 SD[9, 10]). A significant inverse relationship was identified ($r = -0.34$; $p < 0.03$), and it was congruent with the reference range.

Fig. 5 illustrates the relationship of mean platelet volume versus platelet distribution width and compares this range with the reference range for a normal nonpregnant population (data expressed as mean ± 2 SD[11]). A significant direct relationship ($r = 0.19$; $p < 0.03$) was identified, and it differed remarkably from the reference range.

Comment

The data indicate that the mean platelet count does not differ significantly from the second to third trimester, which is consistent with other longitudinal studies during this interval. Although the mean platelet volume did not differ significantly, a highly significant increase in platelet distribution width was discerned during this interval. The findings of this study are in partial agreement with the cross-sectional data of Fay et al.,[4] which demonstrate a significant decrease in platelet count and an increase in mean platelet volume and platelet distribution width with gestational age. These authors interpreted their data to indicate platelet "hyperdestruction" throughout normal pregnancy. Since the mean platelet count did not change significantly, we interpret our data to indicate a compensated state of progressive platelet consumption during normal pregnancy. Numerous investigations support the concept of normal pregnancy as a compensated state of subclinical coagulopathy and suggest maintenance of the uteroplacental interface as its focus. Among the most recent, Rakoczi et al.[12] demonstrated in a limited sampling that platelet half-life appears shorter in pregnancy in comparison with nonpregnant control subjects. β-Thromboglobulin, a platelet-specific release

protein, was found by Douglas et al.[13] to be significantly elevated during the second and third trimesters in comparison with first-trimester values, the values of patients taking oral contraceptives, and the values of nonpregnant control subjects. Further, measurements of soluble fibrin complexes, which are increased in thrombosis and disseminated intravascular coagulation, were found to be significantly increased by the second gestational month and to increase steadily to five times control values by term.[14]

The relationship of mean platelet volume to platelet count was congruent with Bessman's nomogram[9, 10] for normal nonpregnant populations; however, our mean was elevated. The relationship of mean platelet volume to platelet distribution width differed remarkably from Bessman's nomogram[10] for nonpregnant individuals but is not conceptually inconsistent with it. Our data suggest an elevated level of thrombocytopoiesis, which is consistent with a compensated state of progressive platelet consumption that differs from that in the normal population observed by Bessman only by the degree of thrombocytopoiesis implied. This conclusion is based on extrapolations of current concepts of platelet volume behavior in health and disease.[9-11] Bessman and others have demonstrated the following: (1) The mean platelet volume remains unchanged to mildly elevated in compensated states of platelet consumption, that is, chronic immune thrombocytopenia; (2) in severe uncompensated states of platelet consumption, that is, acute immune thrombocytopenia and disseminated intravascular coagulation, mean platelet volume increases markedly and proportionately with the demand for platelet production; (3) in conditions stimulating thrombocytopoiesis, increases in platelet distribution width would be expected to accelerate when mean platelet volume increases because increasing stimulation to platelet production broadens the distribution of platelet sizes produced and affects the platelet distribution width more than the mean platelet volume. Our data demonstrate a mildly elevated mean platelet volume and a greater unit increase in platelet distribution width versus mean platelet volume in comparison with Bessman's nomograms[10, 11] (Figs. 4 and 5). We interpret these data to represent an increased level of thrombocytopoiesis, which is the result of both the dilutional and the consumptive stimuli of normal pregnancy.

These findings may contribute to the understanding of the pathophysiology of thrombocytopenia in preeclampsia. In comparison with normal pregnant control subjects, patients with preeclampsia exhibit a statistically significant decrease in platelet half-life, as well as increases in plasma levels of β-thromboglobulin and soluble fibrin monomers.[12-14] Additionally, platelet consumption has been shown to be an early feature of preeclampsia, which in approximately 20% of patients with severe preeclampsia results in thrombocytopenia, the most common hemostatic abnormality observed in patients with preeclampsia.[15] Further, Giles and Inglis[16] have shown that the platelet count is significantly lower and the mean platelet volume significantly higher in patients with preeclampsia in comparison with normal control subjects or in nonpreeclamptic patients with hypertension. Taken together, these observations suggest that preeclampsia may be associated with an acute coagulopathic state that precedes the clinical onset of disease. Since changes in mean platelet volume and platelet distribution width may precede changes in platelet count and may reflect alterations in the bone marrow environment even in the absence of significant changes in the peripheral platelet count, i.e., compensated states, we suggest that these indices may have both diagnostic and prognostic value in gestational hypertensive states. The occurrence of accelerated platelet consumption in preeclampsia, coupled with low cost and availability of quantification of mean platelet volume and platelet distribution width on automated instruments (with Food and Drug Administration approval), may justify antenatal screening.

In summary, platelet distribution width, mean platelet volume, and platelet count and their interrelationships have now been evaluated longitudinally in pregnancy. These baseline data suggest that uncomplicated pregnancy results in a chronic, progressive state of platelet turnover. Since preeclampsia may be associated with platelet consumption beyond normal gestational demands, we suggest that quantification of platelet indices may have value in screening for and following this disorder. A prospective study is being designed to expand the data base and to apply it to the investigation of gestational hypertension.

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Aortic function during normal human pregnancy

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Arterial rupture and dissection have been observed in both normal and complicated pregnancies. To understand how arterial mechanical properties might change during pregnancy, we measured aortic root diameter by M-mode echocardiography and obtained simultaneous cuff blood pressures during systole and diastole at rest and during isometric exercise. Measurements were obtained in 19 women at the end of normal gestation and again 3 to 6 months post partum. The aorta is larger and more compliant during normal human pregnancy, and these changes may not revert to prepregnant levels post partum. Increased aortic compliance, combined with decreased vascular resistance, may enhance left ventricular performance in normal pregnancy. Conversely, the presumed structural alterations leading to increased compliance may weaken diseased vessels leading to dissection or rupture during pregnancy. (AM J OBSTET GYNECOL 1986;154:887-91.)

Key words: Pregnancy, aortic compliance, aortic size

Rupture of the aorta or one of its branches is an important but infrequent source of maternal morbidity and mortality.[1] While coarctation and Marfan's syndrome[2] are common predisposing factors to these catastrophes, many pregnant women have no apparent cause for arterial rupture. The assessment of maternal aortic structure and function is therefore an important topic. However, information is meager and conflicting regarding whether aortic structure, assessed by light microscopy, is altered during pregnancy.[3, 4]

Even less information is available regarding aortic function during pregnancy. Arterial diastolic pressure declines during pregnancy, a change which should be associated with decreased aortic diameter. However, an echocardiographic study of women throughout pregnancy demonstrated a consistent increase in aortic diastolic diameter during gestation while diastolic blood pressure decreased.[5] Although significant differences were not noted, the data were suggestive of a change in aortic pressure-dimension relationship during pregnancy. However, the stress-strain relationship of rabbit aortic strips in vitro was not affected by pregnancy.[6]

A shift of the aortic pressure-dimension relation would suggest pregnancy-related changes in aortic function and structure and a mechanism for the apparent aortic fragility during gestation. Great vessel mechanics also significantly affect ventricular function.[7] If present, increased aortic compliance would reduce ventricular afterload and enhance left ventricular performance. The goal of the present study was to estimate aortic pressure-dimension relations during and after pregnancy. Measurements were made at several aortic pressures in order to avoid possibly confounding spontaneous pressure changes.

Methods

Subject selection. Study subjects were recruited from consecutive admissions to the Labor and Delivery Service of the Oregon Health Sciences University Hospital. Patients were excluded from the study for any of the following reasons: history of cardiovascular disease, medical complications during pregnancy, multiple pregnancy, advanced stage of labor, or refusal or

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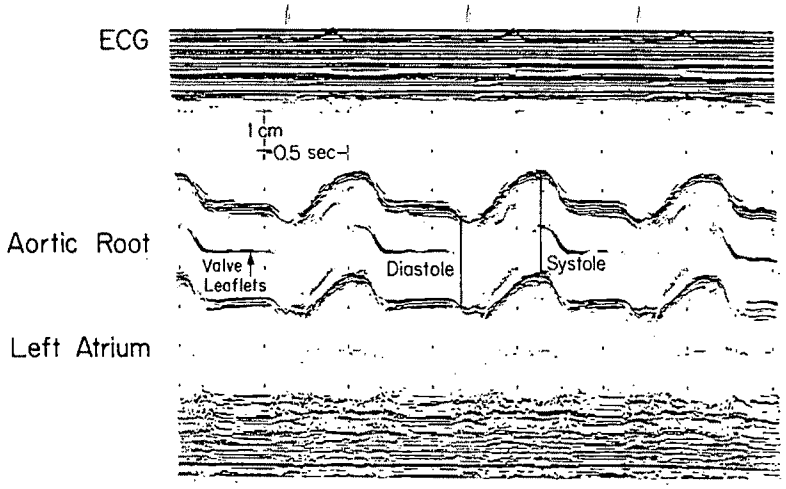


Fig. 1. M-mode echocardiographic display of the aorta during three consecutive cardiac cycles. The aortic valve leaflets are clearly visualized when closed during diastole in the middle of the aortic root, thus providing a consistent landmark for subsequent measurements.

Table I. Maternal and fetal demographic characteristics

	All	*Primiparous* ($n = 7$)	*Multiparous* ($n = 12$)
Fetal weight (gm)	3708 ± 456	3628 ± 567	3810 ± 413
Prepregnant body (m^2)	1.62 ± 0.13	1.68 ± 0.14	1.59 ± 0.12
Gestational weight (kg)	76 ± 12	81 ± 13	73 ± 11
Maternal age (yr)	25 ± 6	23 ± 8	26 ± 5
Gestational age (wk)	40 ± 2	40 ± 2	40 ± 1
Time of postpartum study (wk)	15 ± 7	13 ± 6	16 ± 8
Lactating	10/19	3/7	7/12

inability of the subject to return for a postpartum examination. No subjects were taking medications with cardiovascular activity. After written informed consent, all subjects underwent identical study protocols at the end of gestation and again 3 to 6 months post partum. This investigational protocol was reviewed and approved by the institutional committee on human research.

Study protocol. After resting in the left lateral recumbent position for 10 minutes, M-mode echocardiograms and electrocardiograms were recorded with a Smith-Kline Echoline 20A ultrasonoscope and a strip recorder as previously described.[8] No measurements were made during uterine contractions. Blood pressures were measured simultaneously by sphygmomanometer and auscultation. A 2.25 MHz transducer was positioned in the third or fourth intercostal space to obtain left ventricular measurements. The transducer was then angled superiorly and medially in order to visualize the aortic root in a plane that intersected the aortic valve leaflets during diastole (Fig. 1). Aortic measurements were determined by means of the leading edge technique from the average of five cycles during end diastole (onset of QRS) and end systole (aortic valve closure).[9] In order to provide aortic measurements over a range of pressures, subjects performed isometric exercise with a hand dynanometer and aortic dimensions and blood pressures were obtained at 1-minute intervals until the subjects ceased exercise because of fatigue (3 to 5 minutes at 50% maximum strength).

In order to assess aortic compliance, aortic cross-sectional area (A) was calculated from each measured dimension (D), assuming the aorta to be a cylinder with a length indifferent to pressure ($A = \pi D^2/4$). Cross-sectional area was normalized for prepregnant body surface area in order to account for individual variability related to body size, independent of pregnancy.

The relationships between aortic pressure and cross-sectional area were analyzed by analysis of covariance to determine pregnancy-related changes in slope and cross-sectional area. The reduced data are presented as slopes and y-adjusted means. All other hemodynamic and echocardiographic data were analyzed by paired t test for comparisons between pregnant and post partum states. Demographic data were tested by unpaired t test.

Results

The study group consisted of 19 women who were 15 to 35 years of age (mean = 25) and who had normal

Table II. Resting hemodynamic and echocardiographic data during pregnancy and postpartum period

	End gestation			*Postpartum period*		
	All	*Primiparous (n = 7)*	*Multiparous (n = 12)*	*All*	*Primiparous (n = 7)*	*Multiparous (n = 12)*
Heart rate (beats · min^{-1})	80 ± 14*	72 ± 9†	85 ± 14*	67 ± 9	63 ± 7	69 ± 8
Systolic blood pressure (mm Hg)	115 ± 11	118 ± 11	114 ±	107 ± 8	105 ± 6	109 ± 9
Diastolic blood pressure (mm Hg)	70 ± 10	71 ± 7	69 ± 12	70 ± 7	70 ± 6	70 ± 8
Aortic area (cm^2 · m^{-2})	4.56 ± 1.31‡	3.78 ± 0.75‡	4.94 ± 1.37*	3.49 ± 0.75	3.26 ± 0.63	3.63 ± 0.78
Aortic compliance	0.120‡	0.050†	0.110‡	0.056	0.040	0.058

Values expressed are means ± SD.
*Significance of difference between values at end gestation and postpartum period: $p < 0.01$.
†Significance of difference between values at end gestation and postpartum period: $p < 0.05$.
‡Significance of difference between values at end gestation and postpartum period: $p < 0.005$.

term pregnancies. Seven of the women were primiparous and 12 multiparous (Table I). Table II depicts hemodynamic and echocardiographic data at end gestation and late post partum. Heart rate, normalized mean aortic root area, and compliance were all greater at end gestation than post partum.

A plot of aortic pressures both at rest and during exercise versus aortic cross-sectional area is shown in Fig. 2. The correlation coefficients for the pregnant and postpartum groups are $r = 0.44$ ($p < 0.01$) and $r = 0.67$ ($p < 0.001$), respectively. Analysis of covariance shows that the slope of the aortic pressure-area relation of subjects in late gestation is significantly less ($p < 0.005$) than that of the same women post partum (Table II). Since this regression coefficient (slope) is the inverse of compliance, we interpret these data to mean that the aortic root is more compliant during the third trimester of pregnancy than post partum.

The effect of parity on aortic size and compliance is shown in Fig. 3, *A* and *B*. There was a significant difference in mean aortic cross-sectional area (analysis of covariance) between multiparous and primiparous women, both during pregnancy (4.94 ± 1.31 versus 3.78 ± 0.75 cm^2/m^2, $p < 0.001$, respectively) and post partum (3.63 ± 0.78 versus 3.26 ± 0.63, $p < 0.025$, respectively). Aortic compliance was significantly greater in multiparous than primiparous women during late gestation; however, compliance was not different between the two groups post partum.

Comment

M-mode echocardiography provided a technique for assessing aortic pressure-dimension relationships during human pregnancy that was safe and acceptable to volunteers. Furthermore, M-mode measurements of the human aortic root accurately reflect angiographically determined dimensions over a wide range of aortic size.[10] The timing and amplitude of brachial arterial pressures determined by auscultation differ from those in the ascending aorta, but the differences are not of sufficient magnitude to influence the conclusions of this study. It is important to note that we measured aortic dimension at end systole and not at the time of peak systolic pressure. Our primary concern was that an accurate and reproducible measurement of systolic aortic diameter be obtained. Inspection of Greenfield and Patel's direct measurements of human aortic pressure-dimension relations[11] shows that aortic dimension changes insignificantly between maximum excursion and aortic valve closure. Last, estimates of aortic diameter by echocardiogram would be falsely increased if images were obtained more tangentially during pregnancy because of changes in position of the heart and great vessels. The frontal electrical axis shifts about 15 degrees to the left during pregnancy but little is known about the position of the heart or great vessels.[12] In our experience, the aorta is not well visualized with M mode when viewed tangentially. Finally, an angle greater than 30 degrees from perpendicular would be necessary to explain our results during pregnancy. For all of these reasons, we believe that our pressure-dimension measurements of the aorta give an accurate assessment of aortic size and compliance.

In an earlier serial echocardiographic study, Katz et al.[5] measured several parameters during pregnancy, including aortic root diameter and blood pressure. They found a significant decrease in diastolic blood pressure during the third trimester when compared with postpartum values; a nonsignificant increase in aortic root diameter was observed during the second and third trimesters compared with postpartum values. Our data confirm this trend of increasing aortic diameter during pregnancy, but we did not find a decreased diastolic blood pressure at end gestation. Other observers have noted that diastolic pressure falls in the second trimester and returns to postpartum values in the third trimester.[13] Our data, therefore are entirely compatible with those of Katz et al.[5] However, because

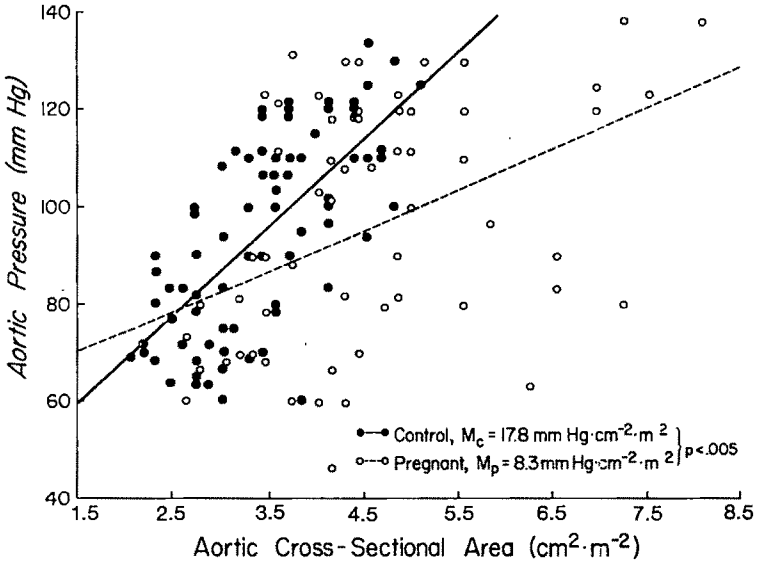


Fig. 2. Aortic pressure–cross-sectional area relationship during pregnancy and post partum. The slope (inverse of compliance) is less during pregnancy, indicating that compliance is greater during this period.

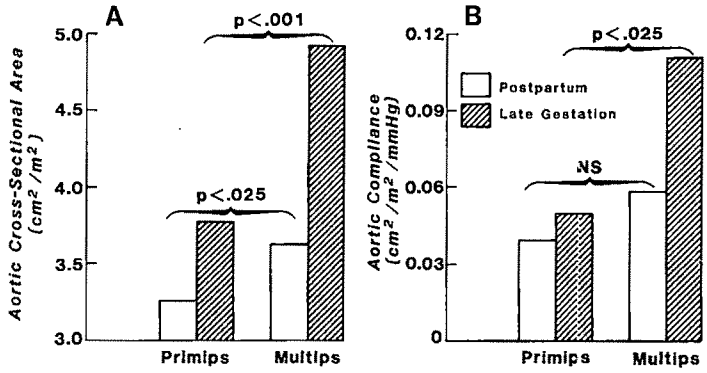


Fig. 3. The effect of parity on aortic cross-sectional area *(A)* and aortic compliance *(B)*. Compared with primiparous women, multiparous women had larger and more compliant aortas during pregnancy. By 3 months post partum, the aortas of multiparous women were still larger than those of primiparous women, but compliance was not different. Aortic cross-sectional area and compliance were greater during pregnancy than post partum for both groups (see Table II).

we examined the aortic pressure-dimension relationship in each subject at several pressures, we are able to offer conclusions regarding the effect of pregnancy on aortic function.

This study was not designed to elicit the underlying mechanism by which aortic mechanical properties are altered during normal pregnancy, but several factors may be involved and are worthy of consideration. Material property changes of the aorta could account for our findings during pregnancy, but only conflicting data concerning the cellular alterations seen in aortic walls during pregnancy are available.[3, 4] Blood volume is increased 40% at term in normal human pregnancy and acute volume loading in dogs increases aortic dimension and compliance.[14] The effect of volume loading on aortic function was noted to be independent of autonomic tone and arterial pressure. However, α-adrenergic tone also affects aortic function.[15] Reduced adrenergic tone in pregnancy has been suggested, but we are unaware of studies that have assessed adrenergic stimulation of aortic smooth muscle during pregnancy.

It appears that sex steroids may play a prominent role in aortic alterations during pregnancy through both direct and indirect effects. It has been noted that several species, including primates, possess specific high-affinity receptor sites for estrogen in aortic tissue.[16] Beldekas et al.[17] have demonstrated that estradiol alters the biosynthesis of collagen in aortic smooth muscle cells by decreasing collagen production and altering the ratio of type I to type II procollagen fractions. Estrogen may also be partly responsible for the large increase in blood volume during pregnancy and blood

volume may affect aortic function as discussed previously. Finally, recent studies have shown that estradiol stimulates the production of prostaglandin cyclooxygenase and prostacyclin synthetase in aortic smooth muscle cells.[18] This is of particular interest as Paller[19] has demonstrated a decreased pressor response to angiotensin II, norepinephrine, and vasopressin during pregnancy in rats. He showed that the blunted pressor response was not due to a change in affinity or number of smooth muscle receptors and that this response could be reversed by administration of a prostaglandin synthesis inhibitor. These observations suggest that the blunted pressor response during pregnancy is mediated by an estrogen-induced increase in prostaglandin synthesis.

We found that multiparous women had larger aortas than primiparous women both during pregnancy and post partum, but aortic compliance was not different between the two groups post partum. These data suggest that some of the alterations of aortic pressure-dimension relations do not revert to the prepregnant state after delivery or return more slowly in multiparous women than in primiparous women.

Increased aortic compliance importantly affects ventricular function. During normal pregnancy in humans and guinea pigs, the left ventricle enlarges in association with an increased stroke volume.[5, 20] However, the ratio of left ventricular radius to wall thickness is increased, a change that would increase wall stress and afterload if aortic impedance were unchanged. The reduced vascular resistance that occurs in pregnancy, combined with the increased aortic compliance reported here, reduces impedance in normal human pregnancy and may explain the preservation of left ventricular shortening velocity noted by Katz et al.[5]

Our findings suggest that aortic structure is altered during human pregnancy and that structural changes explain both the increased aortic dimension and compliance and the unfortunate tendency for aortic fragility during pregnancy. Definitive studies regarding this hypothesis are not available. Biochemical and functional changes in the uterine arteries during pregnancy are well documented and sex steroids, especially estrogen, may play an important role in these changes.[21] These studies need to be extended to the aorta and the role of sex steroids in this process needs to be determined.

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Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings

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The ovarian ultrasonic appearance in 20 patients with polycystic ovarian disease was studied and correlated to the clinical, hormonal, and laparoscopic findings. Ultrasound studies showec that both ovaries were enlarged in 15 patients (15.46 ± 2.5 cm^3). Maximum ovarian surface area was 9.75 ± 3.38 cm^2. Three ultrasonic patterns were detected: (1) isoechoic, with no discernible cysts (four patients); (2) hypoechoic, with multiple small cysts of <1 cm (11 patients); (3) hypoechoic, with single cyst of >1 cm (five patients). Ultrasonic estimation of ovarian size was superior to clinical assessment and equal to that of laparoscopic examination. Subtle differences existed between the ultrasonic appearance of the ovaries in hyperprolactinemic subgroups of polycystic ovarian disease compared to normoprolactinemic ones. However, no significant relationship was found between the ovarian size and any of the hormones studied. Obesity, amenorrhea, hirsutism, hyperprolactinemia, and elevated testosterone and dehydroepiandrosterone sulfate levels were more common in the group with enlarged ovaries, whereas oligomenorrhea, elevated luteinizing hormone/follicle-stimulating hormone ratio, and elevated androstenedione and estrone levels occurred more frequently in the group with normal-sized ovaries. The value of ultrasound studies in the management of polycystic ovarian disease is emphasized. (Am J Obstet Gynecol 1986;154:892-5.)

Key words: Ultrasound, polycystic ovaries, prolactin

Polycystic ovarian disease presents a spectrum of overlapping ovarian and adrenal abnormalities with variable clinical manifestations. The diagnosis of polycystic ovarian disease is sometimes elusive and requires visualization of both ovaries. The first ultrasonic observation of enlarged ovaries in patients with the disease were those of Kratochwil et al.[1] and Zemlyn.[2]

Several reports[3-6] have recently appeared on the sonographic morphology of polycystic ovaries. The ultrasonic diagnosis of polycystic ovaries in many of these studies was not confirmed by hormonal assay or laparoscopy and most of these reports did not correlate the ultrasonic appearance of PCO to the clinical, hormomal and laparoscopic findings.[6]

The aim of this study was to review the ultrasonic appearance of polycystic ovaries and to correlate it to the different clinical, hormonal, and laparoscopic findings of polycystic ovarian disease.

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Material and methods

Twenty patients with PCOD (18 to 35 years) were thoroughly examined clinically, and their findings were recorded. None of the patients received hormonal treatment for at least 3 months before the study. Clinical findings included secondary amenorrhea (45%), oligomenorrhea (35%), primary infertility (94%), hirsutism (70%), obesity (80%), galactorrhea (35%), and acne (15%). Ovarian size as detected by pelvic examination was recorded.

Repeated basal plasma samples were taken at 8 AM under basal conditions and assayed with use of standard radioimmunoassay kits for the following hormones: luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol-17β, estradiol-17β, estrone, progesterone, testosterone, androstenedione and dehydroepiandrosterone sulfate. Each sample (12 ml) was the aliquot of three smaller samples taken at 30-minute intervals. Samples were taken daily for three consecutive days during each of the follicular, periovulatory, and luteal phases of the baseline cycle.

Pelvic ultrasound examination was performed with use of a 3.5 MHz sector real-time transducer (Emisonic 4200). After visualization of the ovaries with use of the full-bladder technique and with the ovarian artery as a landmark, the following sonographic findings were recorded: (1) ovarian size (½ × length × width ×

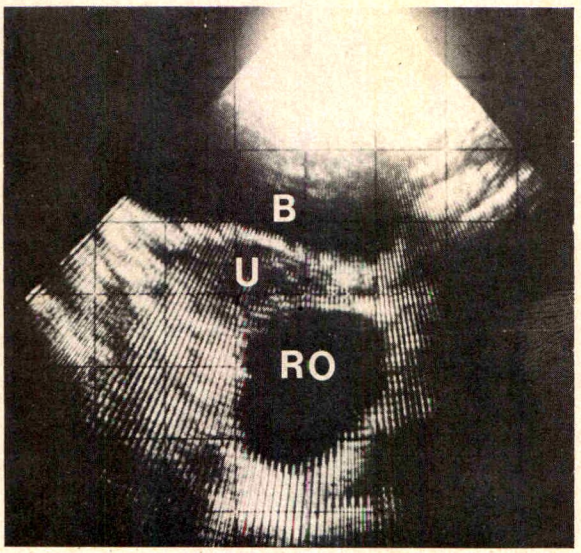


Fig. 1. Oblique scan showing enlarged right ovary (single cyst). *B*, bladder; *RO*, right ovary; *U*, uterus.

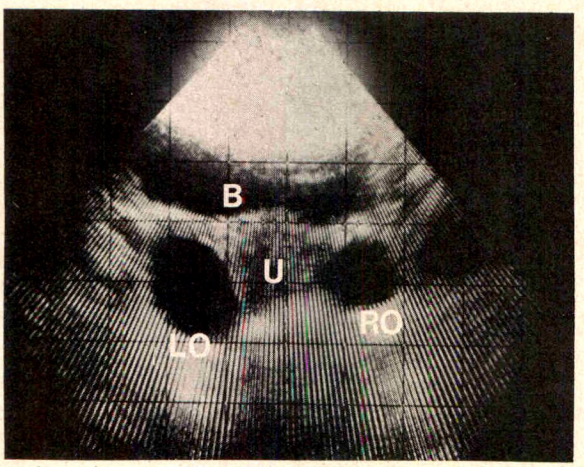


Fig. 2. Transverse scan showing bilaterally enlarged cystic ovaries (single cyst). *B*, bladder; *RO*, right ovary; *LO*, left ovary; *U*, uterus.

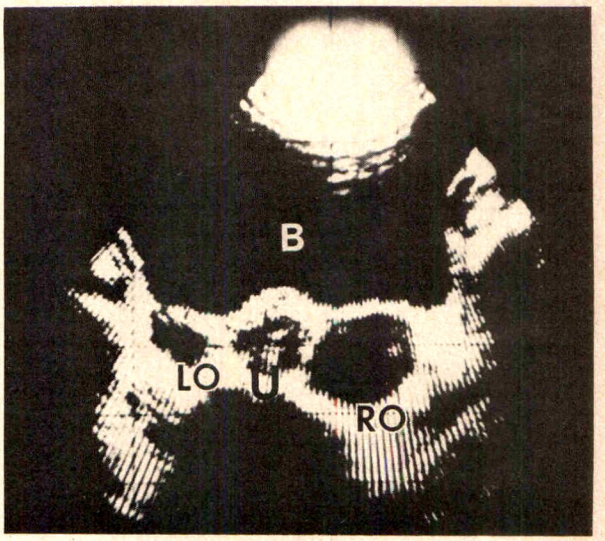


Fig. 3. Transverse scan showing bilaterally enlarged ovaries (more on the right side) with intraovarian trabeculae demonstrating multiple small cysts. *B*, bladder; *RO*, right ovary; *LO*, left ovary; *U*, uterus.

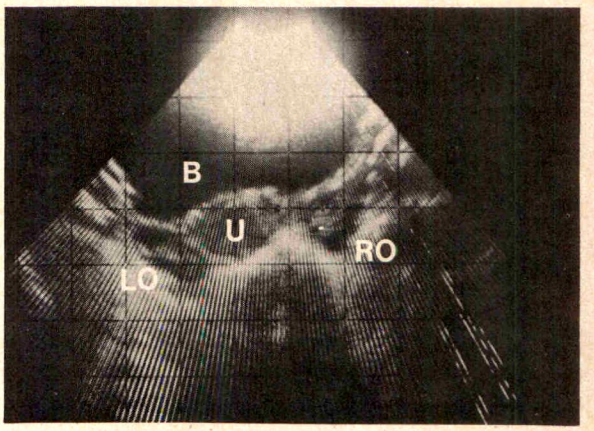


Fig. 4. Transverse scan showing bilaterally enlarged isoechoic ovaries. *B*, bladder; *RO*, right ovary; *LO*, left ovary; *U*, uterus.

thickness)[6]; (2) maximum surface area (maximum diameter × minimum diameter × 0.785)[6]; (3) ovarian echogenicity; (4) presence or absence of ovarian cysts, their number, and size; (5) ovarian margin, whether regular or irregular; (6) ovarian capsule, whether thick or thin.

All sonograms were performed early in the cycle in oligomenorrheic patients or at random in amenorrheic ones. To differentiate normally developing graafian follicles in normal ovaries from follicles in normal-sized polycystic ovaries, repeat scans were sometimes performed. Follicles in polycystic ovaries are bilateral and persist on serial examinations, since ovulation does not occur, whereas normal ovaries will show regression of follicles and development of a corpus luteum on scans obtained later in the menstrual cycle.

Laparoscopy was performed as part of an infertility workup and the ovarian size, shape, capsule, and surface were recorded.

Results

The ovaries were palpable in eight patients (40%) by careful vaginal examination of the unanesthetized patient.

Ultrasound findings. Both ovaries were enlarged in 15 patients (75%) with a mean ovarian size of 15.46 ± 2.5 cm^3 and a range of 12.94 to 20.28 cm^3 (average normal ovarian size is 7.8 ± 0.4 cm^3). The ovaries were of normal size in five patients (5%), with a mean ovarian size of 7.93 ± 0.49 and a range from 6.37 to 7.5 cm^3. The maximum ovarian surface area of the group studied was 9.75 ± 3.38 cm^2 with a range from 6.37 to 20.28 cm^2. The ultrasonic appearance of polycystic ovaries (Figs. 1-4) showed one of three pat-

Table I. Ultrasonic characteristics of polycystic ovarian disease subgroups

Polycystic ovarian disease subgroup	*Ovarian size (cm^3)**	*Enlarged ovaries*		*Isoechoic ovaries*		*Hypoechoic ovaries*		*Multiple small cysts <1 cm*		*Single cyst >1 cm*	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Hyperprolactinemic† (n = 9)	14.36 ± 3.38	7	77.8	1	11	8	89	6	67	2	22
Normoprolactinemic (n = 11)	12.49 ± 5.04	8	72.7	3	27.3	8	72.7	5	45.5	3	27.2

* Mean ± SD.

† Prolactin (mean of three samples) >20 ng/ml.

terns: (1) isoechoic, with no discernible cysts (four patients); (2) hypoechoic, with multiple small cysts of <1 cm in diameter (11 patients); (3) hypoechoic, with single cyst of >1 cm in diamter (five patients).

The ovarian margin was regular in 17 patients (85%) and irregular in three patients (15%). Ovarian capsule was thick (lumpy and echogenic) in 18 patients (90%).

Basal hormonal evaluation showed anovulatory luteal-phase progesterone in all patients (100%), elevated luteinizing hormone/follicle-stimulating hormone ratios in 16 patients (80%), elevated testosterone levels in 16 patients (80%), elevated androstenedione levels in 15 patients (75%), normal or low estradiol-17β levels in 16 patients (80%), high estrone levels in seven patients (35%), and high dehydroepiandrosterone sulfate levels in three patients (15%) as compared to normal control values. Nine patients (45%) were hyperprolactinemic (basal prolactin >20 mg/ml), and 11 patients (55%) were normoprolactinemic.

There was no significant relationship between the ultrasonic ovarian size and any of the hormones studied. The hyperprolactinemic subgroup of polycystic ovarian disease showed larger ovarian size compared to the normoprolactinemic subgroup, but the difference (Table 1) was not statistically significant.

Comparison between patients with polycystic ovarian disease with normal-sized versus enlarged ovaries showed that obesity, amenorrhea, hirsutism, hyperprolactinemia, elevated testosterone and dehydroepiandrosterone sulfate levels, and normal or low estrone levels occurred more often in the group with enlarged ovaries (93% vs. 40%, 46.7% vs. 40%, 80% vs. 60%, 46.7% vs. 40%, 86.7% vs. 60%, 20% vs. 0%, and 86.7% vs. 60%, respectively). On the other hand, oligomenorrhea, elevated luteinizing hormone/follicle-stimulating hormone ratio, and elevated androstenedione and estrone levels were more common in the group with normal-sized ovaries (40% vs. 33.3%, 100% vs. 73.3%, 100% vs. 66.7%, and 60% vs. 26.7%, respectively).

Laparoscopic findings. The uterine size, surface, and mobility were normal in all patients. The tubes looked normal and were bilaterally patent in 19 patients. One patient had evidence of salpingitis with unilateral fimbrial obstruction and peritubal adhesions.

The ovaries showed no signs of ovulation in all patients (100%). Both ovaries were enlarged with a smooth surface and thick white capsule in 15 patients (75%). The ovaries were normal in size with a sclerocystic surface in five patients (25%). Laparoscopy confirmed ultrasonic findings regarding ovarian size, surface, and capsule in all patients.

Comment

This study is one of few reports on the accuracy of ultrasonic diagnosis of polycystic ovaries that were confirmed by hormonal and laparoscopic criteria. Hann et al.[3] studied the ultrasonic appearance of 28 patients with polycystic ovarian disease. He found that 71% of the cases had bilaterally enlarged ovaries, which is close to our findings. The mean ovarian size in his case was 14 cm^3. Thirty-nine percent of his cases showed discrete cysts, 25% were hypoechoic, and 7% were isoechoic to the uterus.

Parisi et al.[6] studied the ultrasonic appearance of 26 patients with polycystic ovarian disease, seven of which cases were confirmed histologically either by surgery or by laparoscopy. The maximum ovarian surface area in their study ranged from 12.9 cm^2 to 17.3 cm^2 with an average of 12.9 ± 2.6 cm^2. In 19 of their patients the ovarian structure was made of many small cystic spaces, approximately 0.5 cm in diameter. In two other cases a cyst of 1.5 cm in diamter was present inside one ovary. In the remaining five patients the ovarian structure was formed of many thick echoes arranged along parallel lines. Parisi et al.[7] found that the edges of polycystic ovaries were lumpy and echogenic. Orsini et al.[8] observed four ultrasonographic ovarian patterns in polycystic ovarian disease: (1) enlarged cystic, (2) enlarged solid, (3) normal-sized cystic, (4) normal-sized solid.

In our study, clinical examination showed enlarged ovaries in only eight of 15 patients proved to have en-

larged ovaries by ultrasound. This emphasizes the superiority of ultrasound in estimating ovarian size in polycystic ovarian disease.

This study showed that regarding ovarian morphology, ultrasound studies were equally as informative as laparoscopic ones. The noninvasive character of ultrasound makes repeat scans and follow-up of ovarian response to therapeutic measures possible.

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Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios

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Severe oligohydramnios, defined as a condition in which the largest pocket of amniotic fluid measures less than 1 cm in its vertical axis as determined by an ultrasound method, was observed in 113 patients in a population of 15,431 referred high-risk patients (0.7%). In all cases, intervention took place unless there was a recognized structural anomaly or exteme prematurity. Overall gross perinatal mortality was 132.7/1000, and the incidence of major anomaly was 13.3%. With intervention the corrected perinatal mortality rate was 17.7/1000, a rate not significantly different from that observed in the entire population. All end points of perinatal mortality were significantly increased in patients with severe oligohydramnios, in comparison with randomly selected control subjects with normal amniotic fluid. These findings are interpreted to indicate that severe oligohydramnios in a structurally normal fetus is an indication for delivery. (Am J Obstet Gynecol 1986;154:895-900.)

Key words: Oligohydramnios, identification by ultrasound, perinatal outcome

Oligohydramnios has long been recognized as a clinical hallmark of impending severe perinatal compromise. Prenatal estimation of the volume of amniotic fluid, when done by physical examination alone, may be difficult and is frequently inaccurate. Since amniotic fluid and its distribution throughout the uterine cavity is readily identified by ultrasound examination, this objective method of evaluation may be used to estimate amniotic fluid volume and its adequacy. Semiquantitative estimation of amniotic fluid volume by an ultrasound method is a component part of fetal biophysical profile scoring.[1] The purpose of this study was to determine the perinatal outcome with the use of perinatal mortality and selected end points of perinatal morbidity in consecutive patients with a singleton pregnancy, intact membranes, and severe oligohydramnios on the last observation before delivery. These statistics were then compared with similar statistics for the entire referral population and morbidity statistics for a randomly selected subgroup to determine the difference in outcome, if any, between these groups.

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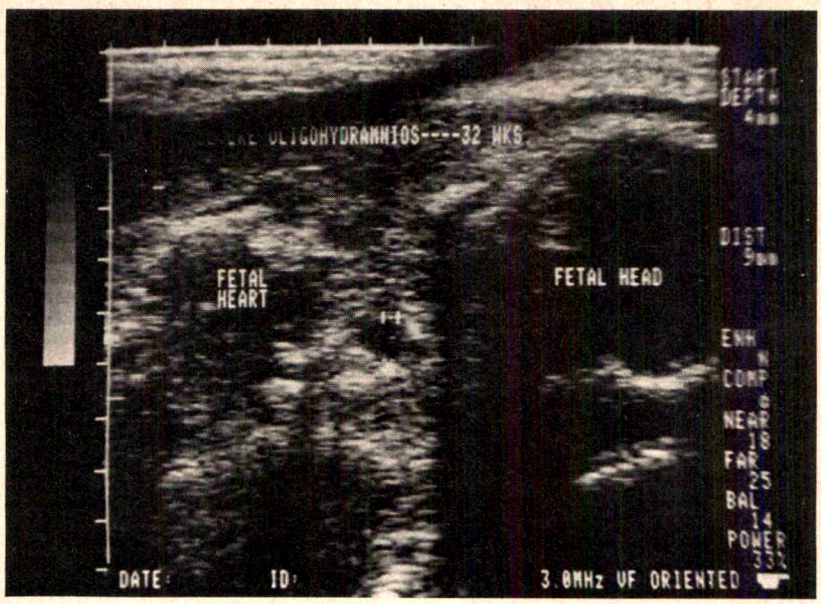


Fig. 1. Real-time B-mode ultrasound scan depicting virtual total absence of amniotic fluid. Note the approximation of uterine and placental tissue directly against fetal structure. This relationship and the absence of any pocket of amniotic fluid were noted at all different planes. This fetus was delivered at 32 weeks and exhibited severe intrauterine growth retardation but was otherwise normal.

Table I. Indications for initial referral for fetal assessment

	Severe oligohydramnios (N = 113)		*Entire referral population (N = 15,431)*		
Primary high-risk factor	*No.*	*%Total*	*No.*	*%Total*	*p Value*
Suspected IUGR	48	42.5	3,209	20.8	NS
Post dates (>42 weeks)	27	23.9	2,469	16.0	NS
Hypertension	13	11.5	2,700	17.5	NS
Maternal medical disease	7	6.2	1,959	12.7	NS
Suspected anomaly	4	3.5	416	2.7	NS
Antepartum hemorrhage	2	1.8	694	4.5	NS
Other	12	10.6	3,984	25.8	NS
Total	113	100	15,431	100	

IUGR = Intrauterine growth retardation; NS = not significant.

Material and methods

Semiquantitative amniotic fluid volume assessment is an integral part of fetal biophysical profile scoring, the primary method of fetal risk determination used at this center.[1] The method involves identification, by dynamic ultrasound, of the largest pocket of amniotic fluid and measurement of this pocket to record the maximal diameter in the vertical plane. Severe oligohydramnios in this study is defined by a generalized decrease in amniotic fluid throughout the uterine cavity such that the largest vertical diameter of any pocket is equal to or less than 1 cm (Fig. 1). In the study period from January 1, 1980, to May 31, 1985 (65 months) the perinatal outcome was reviewed in consecutive patients who had a singleton fetus that was alive at initial presentation, who had intact membranes, and who exhibited severe oligohydramnios. In all patients at all visits, in addition to the recording of amniotic fluid volume, a fetal biophysical profile score was determined and an evaluation for congenital anomalies was done. All patients with severe oligohydramnios were delivered within 48 hours of confirmation of the findings unless a major developmental anomaly incompatible with life was recognized or the gestational age at diagnosis was less than 27 weeks. These results were contrasted with perinatal mortality and the incidence of major anomaly observed in the entire referral population (N = 15,431) and with the end points of perinatal morbidity as determined in a random sample of patients with normal amniotic fluid on the last test before delivery (N = 1000).

End points used to judge perinatal outcome included the following:

1. Perinatal death was defined as the death of a fetus

whose birth weight was 500 gm or more or whose gestational age at death was at least 24 completed weeks or as the death of a live-born infant within the first 28 days of neonatal life. Corrected perinatal death was defined as the death of a structurally normal perinate.

2. Fetal distress was defined as an abnormal fetal heart rate monitoring record either alone or in combination with other abnormal fetal biophysical variables (biophysical profile score <4).

3. Five-minute Apgar score was judged by an independent observer, in most instances either an anesthetist or a neonatologist. A 5-minute Apgar score of <7 was considered abnormal.

4. The incidence of intrauterine growth retardation was defined as a birth weight of less than the tenth percentile for gestational age and sex as indicated by a sea level nomogram.[2]

5. The presence of major structural congenital anomalies.

6. The frequency of admission to an intensive care nursery and the total number of neonatal hospital days were calculated.

Statistical analysis was by nonparametric tests, using χ^2 analysis in most instances. Fisher's exact test was used when a variable frequency of ≤5 was recorded. Differences were considered statistically significant when the p value was equal to or less than 5%.

Population logistics. Semiquantitative amniotic fluid volume determination by an ultrasound method was obtained serially in 15,431 consecutive referred high-risk patients. The study population consists of 113 patients (0.73%) in whom severe oligohydramnios, as defined, was observed on the last test before delivery. Perinatal mortality in the study group was contrasted with the rate observed in the referral population. Perinatal morbidity results in the study group were compared with the same end points obtained in 1000 randomly selected patients with normal amniotic fluid volume observed on the last test before delivery. The primary indications for initial referral to fetal assessment for the entire referred population and the subset with severe oligohydramnios are listed in Table I. No significant difference in indication for referral is noted between these groups, although referral because of a clinical suspicion of intrauterine growth retardation was more than twice as frequent in patients with subsequently proved severe oligohydramnios.

Results

In the 113 study patients the mean maternal age was 26.6 years (range 16 to 47) and the mean parity was 1 (range 0 to 7). The gestational age at delivery ranged from 27 to 44 completed weeks, with most patients (83.2%) delivering at or after 36 completed weeks (Fig. 2). Of 113 patients, 27 (23.9%) were at 42 or more completed weeks at delivery (Fig. 2).

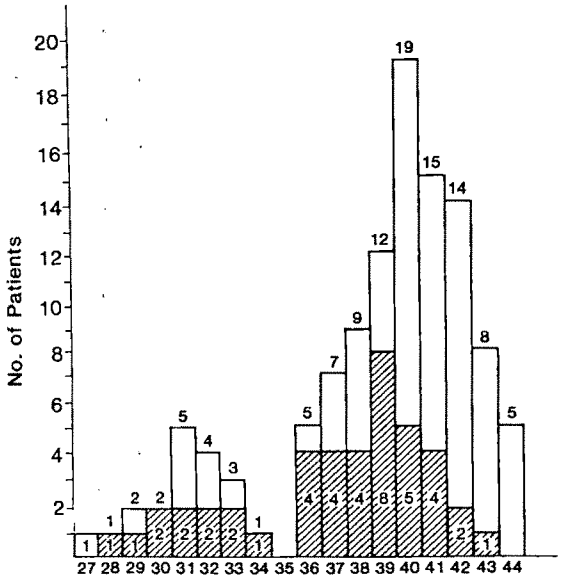


Fig. 2. Distribution of patients with severe oligohydramnios (N = 113) by gestational age at the time of delivery. *Hatched lines*, neonate with intrauterine growth retardation (N = 43).

By protocol, delivery was initiated within 48 hours of recognition of severe oligohydramnios unless there was an associated lethal fetal anomaly complex or extreme prematurity (<27 completed weeks). In 14 patients, delivery was delayed for more than 48 hours and up to 14 days; in 12 of these 14 patients the delay was related to the finding of a lethal anomaly, and in two the delay was due to extreme prematurity. Of 113 patients with severe oligohydramnios, 99 patients were delivered within 48 hours of the last test (87.6%). In 35 of these 99 patients (35.3%) delivery was by cesarean section.

Perinatal outcome. Fifteen perinatal deaths occurred in 113 patients with severe oligohydramnios, giving a gross (uncorrected) perinatal mortality rate of 132.7/1000 (Table II); nine of these 15 deaths occurred as stillbirths. Most stillbirths occurred within 1 week of the diagnosis of oligohydramnios, and the longest interval between diagnosis and death was 11 days. Of the 15 deaths, 13 (86.6%) were due to associated major anomalies: seven fetuses or neonates died of multiple malformations, five of bilateral renal agenesis, and one of a neural tube anomaly. Two other infants with congenital anomalies survived. The corrected perinatal mortality was 17.7/1000 (two deaths). Both of these deaths in structurally normal fetuses occurred in the antepartum period and before 27 completed weeks of gestation (26 and 26.5 weeks, respectively). Since obstetric intervention was not considered before 27 weeks' gestation, these two deaths were considered nonpreventable. Whereas the gross perinatal mortality for patients with severe oligohydramnios (132.7/1000) was significantly higher than the rate observed for the entire referral population (14.2/1000; $p < 0.01$, χ^2 anal-

Table II. Comparative perinatal mortality observed in patients with or without severe oligohydramnios before delivery

PNM variable	Patient population				
	Severe oligohydramnios, treated (N = 113)	*Entire referred population (N = 15,431)*	*p Value**	*Severe oligohydramnios,† nontreated (N = 55)*	*p Value**
Gross (uncorrected) PNM rate per 1000 births	132.7	14.20	<0.01	109.0	NS
Corrected PNM rate per 1000 births	17.7	7.35	NS	89.8	<0.05

PNM = Perinatal mortality; NS = not significant.

*Compared with the study population.

†Data from Manning FA, Platt LD, Sipos L. Antepartum fetal evaluation: development of a fetal biophysical score. AM J OBSTET GYNECOL 1980;136:787.

Table III. Comparative perinatal morbidity statistics in patients delivered with or without severe oligohydramnios

Perinatal morbidity variables	*Severe oligohydramnios* (N = 99)* (%)	*Random control subjects (N = 1000)* (%)	*p Value*	*Prospective nontreated group† (N = 26)* (%)
Fetal distress	38.4	7.8	$p < 0.01$	28.6 (NS)
Low 5-minute Apgar score	15.2	4.0	$p < 0.05$	23.6 (NS)
IUGR (<tenth percentile)	36.5	5.5	$p < 0.01$	—
Major anomaly‡	13.3	0.7	$p < 0.01$	—
Neonatal intensive care admission	43.3	6.5	$p < 0.05$	—

NS = Not significant; IUGR = intrauterine growth retardation.

*Excludes patients with stillbirth.

†Data from Manning FA, Platt LD, Sipos L: Antepartum fetal evaluation: development of a fetal biophysical profile score. AM J OBSTET GYNECOL 1980;136:787.

‡Includes patients with stillbirth.

ysis), the corrected perinatal mortality rate for these two populations (17.7 and 7.35/1000, respectively) did not vary significantly (Table II).

In 99 patients with severe oligohydramnios, perinatal salvage was considered feasible, and for these patients delivery was accomplished within 48 hours of the initial diagnosis. In 38 of these 99 fetuses (38.4%), fetal heart rate monitoring yielded abnormal results either alone or in combination with other abnormal biophysical variables (Table III). This rate of fetal distress was significantly greater than the similar rate observed in the control referred population (7.8%; $p < 0.01$, χ^2 analysis). The rate of fetal distress in patients in whom recognition of severe oligohydramnios prompted obstetric intervention did not vary significantly from previously published results in similar patients managed conservatively[1] (28.8%) (Table III). In 15 of 99 patients (15.2%) the 5-minute Apgar score was less than 7, a rate significantly higher than that observed in the control referral population (4%; $p < 0.05$, χ^2 analysis). Comparison of the incidence of low 5-minute Apgar scores between treated patients with severe oligohydramnios (15.2%) and previously published nontreated cases (23.6%) revealed no significant difference (Table III). Intrauterine growth retardation, defined as a birth weight of less than the tenth percentile for gestational age and sex, occurred in 38 of 104 live-born infants (36.5%), a rate significantly higher than the rate observed for the control referral population (5.5%; $p < 0.01$, χ^2 analysis). Of these 38 fetuses, 25 (65.8%) were suspected to have intrauterine growth retardation on clinical grounds, prompting initial referral. Considered collectively, 15 of 113 patients with severe oligohydramnios (13.3%) delivered a child with a major developmental anomaly. The rate of major anomaly in the control referral population was 0.7%. These differences were highly significant ($p < 0.001$, χ^2 analysis). Of 104 live-born fetuses, 45 (43.3%) required admission to an intensive care nursery, in comparison with 6.5% of the control referral population ($p < 0.01$, χ^2 analysis). The average hospital days for neonates delivered with severe oligohydramnios was 10.4 days (range 2 to 63); comparable statistics for the control referral population were not readily available.

Comment

Severe oligohydramnios, as defined by an ultrasound method in this study, is an unusual finding in referred high-risk pregnancies, complicating only 0.73% of all cases. A similar low frequency (0.85%) of severe oli-

gohydramnios is reported by Chamberlain et al.[3] in a similar population with the use of the same diagnostic criteria. Severe oligohydramnios, despite its infrequency, appears to be an important sign of perinatal compromise. All measures of perinatal mortality and morbidity applied to patients with severe oligohydramnios were significantly increased when compared with rates observed in the entire referral population or the randomly selected control group. Several of these differences warrant further comment. First, the gross perinatal mortality rate was significantly higher among patients with severe oligohydramnios in comparison with the entire referral population, a difference that was due primarily to the increased frequency of lethal malformation among the former group (13.3% and 0.7%, respectively). The corrected perinatal mortality rate, achieved by exclusion of deaths caused by major anomaly between the two groups, did not differ significantly (Table II). In contrast, the corrected perinatal mortality in patients with severe oligohydramnios in whom delivery was initiated (17.7/1000) was significantly lower than the rate reported in a similar population managed conservatively (89.9/1000[1]; Table II). We interpret these findings as indicating that intervention for severe oligohydramnios resulted in an improved probability of perinatal survival. This supposition remains open to challenge because the two groups compared, although subjected to the same diagnostic criteria, come from different institutions and were seen during different periods.

The incidence of low 5-minute Apgar score and those of fetal distress and admission to an intensive care nursery were all sharply and significantly increased in patients with severe oligohydramnios, in comparison with the random control group (Table III). The incidence of low 5-minute Apgar scores in patients delivered because of severe oligohydramnios (15.2%) did not significantly differ from that observed in similar patients managed conservatively (23.6%[1]). Thus, despite intervention in patients with severe oligohydramnios, the probability of low 5-minute Apgar scores remains high, confirming the increased probability of perinatal compromise in such cases. These end points, used to judge perinatal morbidity, although prospectively and in a uniform manner, must be considered "soft" measures of compromise and subject to observer bias. Nonetheless, since the object of perinatal intervention is to reduce morbidity as well as mortality, the increased incidence of these more subjective measurements of perinatal morbidity must be considered in management decisions regarding patients with severe oligohydramnios.

The incidence of developmental structural anomalies and that of intrauterine growth retardation, both more objective measures of perinatal compromise, were considerably increased in patients with severe oligohydramnios. Renal agenesis was observed in 4.4% of patients with severe oligohydramnios; this association may be expected because fetal urine is a major source of amniotic fluid in later gestation. The diagnostic criteria for diagnosis of renal agenesis are well established, including both ultrasound assessment for presence of renal tissue and serial fetal bladder volume determination to confirm normal renal function.[4] Yet pharmacologic tests of fetal urine production must be interpreted with caution.[5] We were surprised to find the relatively high frequency of major anomalies involving organ systems other than the genitourinary tract in patients with severe oligohydramnios (eight cases). The basis for this association remains unexplained.

The incidence of intrauterine growth retardation was significantly increased among patients with severe oligohydramnios (38%, 43 of 113 cases); yet most patients with severe oligohydramnios did not deliver a fetus with intrauterine growth retardation (62%). The combination of a clinical suspicion of intrauterine growth retardation (prompting initial referral for fetal assessment) and documented severe oligohydramnios yielded a true incidence of oligohydramnios of 69.8% (30 of 43 cases). This rate, although high, is less than that reported previously for this association by Manning et al.[6] (90%). These differences almost certainly reflect differences in size-versus-dates discrepancies, prompting initial referral for suspected intrauterine growth retardation. The mechanism by which oligohydramnios is associated with increased frequency of perinatal compromise remains conjectural. Intrauterine fetal hypoxemia and/or asphyxia may cause redistribution of cardiac output away from the fetal lung and kidney, the two major sources of amniotic fluid in later gestation, and therefore may be the cause of progressive oligohydramnios.[7] The observation of a diminished urine production rate, measured indirectly by ultrasound assessment of bladder volume in fetuses with intrauterine growth retardation, supports this contention.[8] In contrast, in some instances, oligohydramnios, produced by whatever mechanism, may ultimately result in cord compression.[9] Therefore, in such cases, oligohydramnios may also be the cause of perinatal compromise. It is likely that in many fetuses with severe oligohydramnios, both mechanisms of perinatal compromise may be operant.

In summary, these data suggest that the antenatal detection of severe oligohydramnios may indicate a high probability of perinatal compromise, together with an increased risk of perinatal death. In such patients, initial assessment must include evaluation of fetal structure with particular, but not exclusive, reference to the fetal genitourinary tract. In the fetus judged to be structurally normal, we interpret these findings to

indicate that obstetric intervention is both warranted and indicated.

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External cephalic version of the breech presentation under tocolysis

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External cephalic version with tocolysis at or near term has been advocated to avoid cesarean birth for breech presentation. In our institution this maneuver was successfully performed in 207 of 304 parturients without major complications, and all but six had vertex presentation at delivery. The success of version was inversely correlated with gestational age but was not correlated with ease of version, number of attempts, or placental location. When this 3-year period was compared with the previous three years (1979 to 1981), there was a significant reduction in the number of breech presentations during labor, whereas the total delivery rate remained relatively constant over the 6-year period. It appears that in a carefully selected population, external version near term can be used safely to reduce the need for abdominal birth because of breech presentation. (Am J Obstet Gynecol 1986;154:900-3.)

Key words: Tocolysis, external cephalic version, breech presentation

It is well known that vaginal delivery in a breech presentation involves a greater risk of perinatal mortality and morbidity than that in a cephalic presentation.[1] The increased damage in breech infants at term frequently appears to be the result of traumatic delivery in cases showing slight degrees of disproportion not easily predicted in advance by clinical or radiologic study.[2] It has been suggested that cesarean birth should be employed more often in an effort to reduce neonatal damage at term with breech presentation, but this strategy increases maternal mortality and morbidity. Two management schemes to address this dilemma have evolved: one embraces routine abdominal delivery for breech presentation, whereas the other advocates scoring systems to select patients for "safe" vaginal delivery.[3, 4] Both management techniques, however, result in an increased cesarean birth rate with its inherently high maternal morbidity rate, and neither attacks the primary problem of the breech presentation itself.

Several authors have advocated early external cephalic version as a simple and safe method to reduce the incidence of breech presentation at term.[5, 6] Unfortunately, the earlier the version attempt, the more

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Table I. Presentation in labor after version

Version attempt	*No. of patients*	*Presentation*		*Method of delivery*	
		Vertex	*Breech*	*Vaginal*	*Abdominal*
Successful	207	201	6	182	25
Unsuccessful	97	0	97	37	60

likely the fetus is to return to the breech presentation, whereas the later the version, the less likely the attempt will succeed.[6] Further, some care providers believe that the procedure may pose excessive fetal risk in the form of placental separation, cord accident, or Rh sensitization.[5]

To circumvent some of these problems, Saling and Muller-Holve[7] proposed the practice of external cephalic version using β-sympathomimetic tocolysis after 37 weeks' gestation. This method offers several potential advantages over early version: (1) spontaneous reversion to breech is uncommon, (2) if fetal distress occurs, the fetus is mature enough for immediate delivery, and (3) uterine relaxation should facilitate easier manipulation. More recently, others have corroborated those findings.[8, 9] Reports on the use of tocolysis in a larger number of patients to access the potential complications to mother and fetus, as well as its impact on the mode of delivery, were not available in the literature. For that reason a study was undertaken in our institution.

Material and methods

During a 3-year period (January, 1982, to December, 1984), 304 normal, "low-risk" patients with breech presentations at greater than 36 weeks' gestation were selected for attempted breech version and agreed to the study protocol, which had been approved by the Institutional Review Board of the University of Mississippi Medical Center. Details of the procedure, potential benefits and risks, and the safeguards to be employed were explained to the patient. The decision to perform the procedure was contingent on her understanding of the protocol. Patients were entered in the study after informed consent forms had been signed. Patients with the following conditions were excluded: (1) maternal cardiac disease, (2) hypertension, (3) premature rupture of the membranes, (4) intrauterine growth retardation, (5) previous uterine surgery, (6) multiple gestation, (7) placenta previa, and (8) oligohydramnios. Requirements for inclusion into the study included (1) gestation ≥36 weeks, (2) breech presentation, (3) absence of any contraindication (exclusion criteria), (4) normal sonographic appearance of fetus, placenta, and amniotic fluid volume, and (5) a reactive nonstress test.

The procedure was conducted in the labor and delivery area. All patients underwent a thorough real-time ultrasound B scanning examination, a nonstress test, and a pelvic examination. A normal real-time B scan was defined as follows: singleton fetus in the breech position; adequate fluid (at least a 1.0 cm pocket of fluid); biparietal diameter >8.5 cm but <10.5 cm and an absence of gross fetal anomalies; or placenta previa. Patients were placed in the lateral semirecumbent position with left uterine displacement. An intravenous line was initiated with a crystalloid solution. A nonstress test was performed, and after a reactive pattern was noted, ritodrine hydrochloride was infused at 0.1 mg/min for 15 minutes prior to and during the procedure.

Maternal heart rate and blood pressure were continuously monitored, as was the fetal heart rate. Version was accomplished by the classic "forward roll" technique if the fetal spine and head were on the same side of the maternal midline. The "back flip" method of Saling and Muller-Holve[7] was used if the initial method failed. A second examiner manipulated the opposite fetal pole or assisted by vaginal elevation of the breech. The procedure was interrupted if patient discomfort became severe or if fetal heart rate decelerations were noted. In many cases, multiple attempts at version were performed. Once the fetus was turned satisfactorily, the β-sympathomimetic infusion was discontinued and the fetus was maintained in the new attitude until uterine tone returned. Real-time B scan was used to confirm version success. For Rh-negative, unsensitized patients, Rh immune globulin was administered following version. After the procedure, fetal heart rate monitoring was continued until a reactive nonstress test was noted.

The following maternal, fetal, and newborn variables were tabulated: (1) ease of version, defined according to the amount of time required to complete the maneuver and the number of attempts (an easy version was completed within 1 minute, with one or two attempts; a moderately difficult version was between 1 and 5 minutes, with two to five attempts; and a difficult version required longer than 5 minutes, with more than five attempts); (2) discomfort during the procedure (patients were asked to describe the procedure as painless or as mildly, moderately, or severely uncomfortable); (3) maternal heart rate and blood pressure; (4) fetal heart rate and uterine activity during and following the version; (5) vaginal bleeding, timing of rupture of the

Table II. Effect of gestational age in version

Gestational age (wk)	Successful*		Unsuccessful	
	n	%	n	%
37-39	179	73	64	27
40-43	28	45	33	55

*p < 0.001.

Table III. Effect of placental location on version

Placental location*	Successful	Unsuccessful	
		n	%
Anterior (n = 128)	83	45	46
Posterior (n = 176)	124	52	54
Total (n = 304)	207	97	

*p < 0.3.

membranes, and onset of labor; (6) intrapartum presentation and route of delivery; and (7) evidence of morbidity during the neonatal period. The χ^2 analysis was used to detect statistical differences, and a p value of <0.05 was considered significant.

Results

Version was successful in 207 of 304 patients (68%). In all of the successful versions, the fetuses had vertex presentations during labor, except six that reverted spontaneously to breech; five of them were delivered by cesarean birth (Table I). A subseptate uterus and a partial placenta previa were found during a cesarean delivery in two of these five cases. The abdominal delivery rate among the subjects (201) with a vertex presentation was 10%, and in no case was the indication for cesarean birth thought to be associated with the version. Of the 97 futile attempts, all deliveries were breech births and 62% were operative births (Table I).

Of the successful versions, the vast majority were deemed easy (167), whereas 31 were moderately difficult and nine were difficult. Of these 207 parturients, 66 patients reported the procedure to be painless, 118 noted mild discomfort, and 23 described the version to be moderately uncomfortable. No patients reported severe or intolerable pain. Among the unsuccessful group, severe pain was the reason to discontinue the procedure in 12 subjects, whereas the type of pain noted subjectively was equally distributed (none, mild, moderate) among the other 85 patients in this group.

In none of the 304 patients did rupture of the membranes, vaginal bleeding, or onset of labor occur within 24 hours of the attempted version. In none of the cases did uterine rupture or catastrophic maternal-fetal events occur. Fetal heart rate abnormalities (tachycardia, bradycardia, change in baseline, variable decelerations) occurred in 18% of the subjects during or after the procedure, but all responded to conservative management (oxygen, change in position, fluid administration with observation). Fetal heart rate abnormalities were a reason to abort version attempts in only six patients and were not related to the number of attempts or the ease of version. In no case was emergency delivery necessary. Likewise, hypotension, tachycardia, or other maternal side effects of the tocolytic agent did not necessitate abandonment of the procedure in any of the 304 patients. The total time for the procedure from entry into the delivery area until discharge was 160 ± 43 minutes among the successful group and 180 ± 51 minutes in those with failed version attempts.

Table IV. Mode of delivery for singleton breech fetuses at term

	1979-1981		1982-1984	
	n	%	n	%
All deliveries	12,942		13,221	
Cesarean birth (breech)	359	61	216	59
Vaginal delivery (breech)	230	39	150	41
Total	589		366*	

*p < 0.001.

A significant difference in version success rate was noted when gestational age at the time of version was compared (Table II). At gestatational ages of 37 to 39 weeks, 179 of 243 attempts (73%) were successful in comparison with 45% (28 of 61) at 40 to 43 weeks (p < 0.001). In contrast, no difference was apparent in version success rate when placental location was used as the discriminator (Table III). Version was unsuccessful in 45 of 128 patients (46%) with anterior placentation and in 52 of 176 patients (54%) with posterior placental placement (p = 0.3).

Careful examination during labor and at delivery disclosed no increased incidence of maternal-fetal-neonatal abnormalities related to the procedure. No increase in meconium-stained fluid, abnormal intrapartum fetal heart rate tracings, low Apgar scores, or neonatal or placental trauma was noted among the total group in comparison with a like number of term infants whose mothers did not undergo an attempted version. In none of the Rh-negative parturients was sensitization noted at delivery.

Comment

The data from this study and a review of the literature leave little doubt that external cephalic version near term will reduce the incidence of breech presentation in labor. Between 90% and 97% of successful versions can be expected to remain as vertex presen-

tations until the onset of labor.[8-10] In addition, few breech fetuses spontaneously convert to the vertex presentation after 36 weeks' gestation.[6] In this study, 68% of the breech presentations were converted to vertex successfully, and all but six fetuses presented in this attitude; figures were similar to those in other series.[7-9]

The impact of breech version on the cesarean birth rate has not been well defined, primarily because of diverse strategies regarding the management of breech presentations at term and because of the lack of large numbers of patients in most studies. We have been challenged to reduce the number of abdominal deliveries.[11] In our study, only 10% of successful versions required cesarean section, in comparison with 60% of the failure group. As shown in Table IV, the number of breech deliveries during the study period was significantly reduced ($p < 0.001$) in comparison with the preceding 3 years, although the total delivery rate increased by 5.4%. The percentage of cesarean versus vaginal deliveries did not differ during both periods for patients with a breech presentation during labor. As a result, the abdominal delivery rate was reduced by this strategy. Obviously, the higher the cesarean birth rate for breech presentation at term in any institution, the greater will be the impact of a program of external version.

The most pertinent question, other than the success of this modality, involves the safety of the procedure.[10, 12] In this study, no intrauterine deaths or neonatal losses attributed to a cord accident or placental abruption occurred. Other studies offer similar results.[8-10] Strict insistence on a normal fetal heart rate pattern both before and after version, as well as a normal real-time B scan, identifies the potentially compromised fetus and/or placental unit. Careful intraprocedure monitoring allows repetitive manipulation in individual cases where fetal heart rate irregularities transiently occur.

In conclusion, these data indicate that in the carefully monitored and selected patient population, external version, near term, of the breech presentation can safely increase the vertex attitude by the onset of labor. This strategy would appear to reduce the need for cesarean birth. Constant vigilance, however, is necessary during the procedure to ensure that one does not damage the fetus or the parturient.

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Prophylactic tocolysis of twins

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The prophylactic use of oral terbutaline was studied in 28 normal, healthy, low-risk women with twin gestations. The mean birth weights were significantly increased over those in a similar group of control patients. (AM J OBSTET GYNECOL 1986;154:904-5.)

Key words: Multiple pregnancy, prophylactic tocolysis

Greater attention is being directed at the twin gestation since it contributes disproportionately to the total perinatal morbidity and mortality in the United States and elsewhere. The most important perinatal factors responsible for this are the significantly higher incidence of premature labor and infants of low birth weight. The risk of pregnancy loss in twin gestation remains three or more times that of singleton pregnancy and respiratory distress syndrome is the most common cause of death.

Early detection of a multiple gestation is a critical first step in any effort to reduce the obvious risks of prematurity. Recently, an increasing amount of attention has been directed at attempting to improve the perinatal outcome in this special group of high-risk mothers. One such logical attempt is the prophylactic pharmacologic reduction of uterine activity.

Although bed rest has been an important part of preventing premature labor, more recent clinical studies[1] have observed that 70% of twin perinatal mortality occurs before the thirtieth week of gestation. Thus if any protocol is to be effective it should be instituted well before this time. It has been emphasized repeatedly that the most effective regimens are those resulting in a decreased incidence of prematurity. Because any preventive measure requires early detection, several authors have suggested more frequent ultrasonographic examinations to detect twins. Once twins are detected, most reports have recommended bed rest as the single most effective treatment for the prevention of premature labor.

The purpose of this prospective study was to determine if an orally administered prophylactic tocolytic agent and modified bed rest regimen would be superior to bed rest alone as a method for the prevention of labor.

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Table I. Case material

	Study	*Control*
No. of patients	28	32
Age (yr)	23.8	25.9
Parity	2.4	2.3
Primigravid	4	2
Multigravid	24	30

Table II. Results

	Study	*Control*
Entry gestation (wk)	21.8	21.4
Time gained (days)	55.6	29.4
Average weight (gm)	2308.8	1937.2

$p = 0.007$.

Case material and outcome

A group of 60 healthy women with low-risk twin gestations detected between 20 and 30 weeks of gestation were included in this prospective study. All patients were of low socioeconomic status and attended the prenatal clinics at University Hospital of Jacksonville. The age and parity distribution was similar in both groups (Table I). Women with complicating factors such as diabetes, prior premature labor, incompetent cervix, and hypertension were not included in the study. After the diagnosis was made all women were advised to stop working and institute maximum bed rest and as much abstinence from intercourse as reasonably possible. Patients were not admitted to the hospital but were followed up in a special twin clinic. Pelvic examinations were rarely performed.

The study group of 28 women were started on a regimen of terbutaline by mouth, 2.5 mg every 6 hours. If uterine activity was noted the interval was shortened to every 4 hours, and rarely the dose was increased to 5 mg. These patients continued on the drug regimen until 35 weeks' gestation. The control group consisted of 32 women treated in a similar fashion who did not receive terbutaline. Although the study was prospective and all patients were treated the same way by the same team it was not possible to blind the medications. Pa-

tients were informed that the drug terbutaline was not approved for this use but was frequently used in this institution for premature labor. The treatment protocol was submitted to the Committee on Human Experimentation and approved.

The time of entry into the study was similar in both groups. The mean length of gestation was longer in the study patients and the additional days the infant was retained in utero was greater for the study patients (Table II).

A *t* test for independent means for birth weights was used. A significant difference in the mean was observed ($p = 0.007$). The standard deviation of the mean was 478.9 in the control group and 554.2 in the study group, while the standard error of the mean was 84.7 and 104.7, respectively.

Although some reports have suggested starting bed rest after the twenty-ninth or thirtieth week, the recent data of Keith et al.[3] show this is clearly too late, as more than 70% of the perinatal mortality associated with twins occurs before the thirtieth week. This would suggest the potential benefit of the early use of terbutaline. This critical period of <30 weeks has been previously alluded to by Manlan and Scott.[4] Hospitalization for bed rest after 30 weeks did not decrease perinatal mortality. Therefore, if hospitalization for bed rest and/or the use of prophylactic tocolysis can be expected to exert any impact on both the survival and the quality of survival of these infants, it must include the 25- to 30-week period.

Little benefit can be expected after 32 weeks. Rather, after this time, intrauterine growth retardation becomes the most important cause of prenatal wastage, constituting 30% in the study of Keith et al. with most such cases presenting as preterm stillbirths. Although bed rest should be encouraged after the thirty-second week, of greater importance is increased attention to antenatal assessment. The place of tocolytics after ≥32 weeks will require further evaluation.

Comment

Having diagnosed twins, the obstetrician is confronted with what preventative measures to institute so as to improve perinatal outcome.

The results of this study show that the use of oral terbutaline combined with bed rest was superior to bed rest alone. The mean duration of gestation was prolonged. Although the perinatal mortality could not be compared because of a small sample size, larger numbers should show a positive effect.

The cornerstone for any plan to improve the management of twin pregnancies must be early detection. It is clear that general screening measures are necessary to improve early detection sufficiently to allow any proposed preventative programs to be effective. Once this is accomplished then the use of prophylactic tocolysis is a logical next step.

To date most studies have focused on the role of bed rest. Many reports[1, 3] have suggested benefit in prolonging pregnancy. Because of the logical basis for bed rest with twin pregnancies, it has come into widespread clinical practice and persisted autonomously, requiring no other justification for its use. The use of oral prophylactic tocolysis may allow a further improvement when combined with the use of bed rest.

In summary, the most significant factor in twin mortality is pregnancy termination before the physiologic maturation of the fetus. Almost half of all the infants will weigh <2500 gm, and the use of pharmacologic agents, β-adrenergic drugs, and intravenous alcohol has not consistently been observed to prolong gestation. In this study bed rest and terbutaline have been shown to be associated with higher birth weights in twins compared with those of twins whose mothers had not received the drug. The mechanism for the increased fetal weight is not yet known, but several factors may be operative, such as improved uterine blood flow and possibly increased fetal glucose levels. The concept of bed rest plus tocolysis is attractive because of its simplicity and low cost. The addition of prophylactic terbutaline to bed rest in 28 women was found to be of value in the management of multiple gestations that were otherwise uncomplicated.

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Epidemiology of hydatidiform mole during early gestation

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Different methodologic approaches have resulted in a wide variability in the reported incidence of hydatidiform mole in the United States. We studied the epidemiology of hydatidiform mole among 84,318 women obtaining legal abortions in the United States during the period 1975 to 1978. Our estimated rate of 7.5 hydatidiform moles per 10,000 pregnancies was similar to most reported rates for the United States. The rate was significantly higher for women 14 years or younger and those 45 years or older. Race, number of previous pregnancies, and number of previous spontaneous abortions were not found to significantly affect the risk of having the disease. The incidence was slightly (but not significantly) higher for women obtaining abortions during the first trimester than for those obtaining abortions during later gestation. This study minimizes the errors encountered in estimating the total number of pregnancies and the number of hydatidiform moles at later stages of gestation. Hydatidiform mole, although relatively uncommon, represents a complication of pregnancy that is of public health importance. (AM J OBSTET GYNECOL 1986;154:906-9.)

Key words: Hydatidiform mole, gestational trophoblastic disease, legal abortion

Hydatidiform mole is an uncommon but important complication of pregnancy. In addition to resulting in the loss of a pregnancy that may be planned and wanted, a hydatidiform mole predisposes a pregnant woman to excessive nausea, vomiting, and malaise.[1] Hydatidiform mole also places a pregnant woman at higher risks of bleeding, pregnancy-induced hypertension, acute pulmonary embolism, and, in rare cases, hyperthyroidism.[2] In 16% of the cases the condition progresses to invasive mole, and in 2.5% it progresses to choriocarcinoma.[2] To prevent the occurrence of the latter two conditions, patients are routinely subjected to repeated laboratory tests, and about 10% of the patients will eventually require chemotherapy.[1]

There has been a wide variability in the reported incidence of hydatidiform mole even within the same country. The reported incidence of hydatidiform mole throughout the world ranges from over 100 per 10,000 pregnancies in Indonesia[3] to 6.9 and 7.7 per 10,000 pregnancies in the United States.[4,5] Within the United States, estimated incidences include 5.8 per 10,000 pregnancies in New York,[6] 9.0 per 10,000 deliveries in California,[7] and 10.8 per 10,000 pregnancies for the United States as a whole.[8] One important reason for this variability in reported incidences is the methodologic approach to assessing the incidence.[9] Part of the problem is the difficulty of assessing the number of women at risk (ideally the number of pregnancies), since that should include live births, still births, ectopic pregnancies, spontaneous abortions, and induced abortions during the study period. Another more important part of the problem is the difficulty in determining the number of cases of hydatidiform mole, since some cases terminate through either spontaneous abortion or induced abortion. Thus a true incidence of hydatidiform mole might more accurately be determined from abortions in early pregnancy.

In this study we review cases of hydatidiform mole among a sample of women undergoing induced abortions in the United States form 1975 to 1978 in an attempt to estimate the incidence of the disease as well as to determine the role of age, race, number of pregnancies, and number of previous spontaneous abortions as risk factors.

Methods

We used information collected by the Joint Program for the Study of Abortion III, a prospective, multicenter, observational cohort study conducted between 1975 and 1978 under the auspices of the Centers for Disease Control to examine early medical complications of legal abortions. This study involved 84,318 abortions performed in 13 institutions. Data were collected by specially trained abstractors at each institution. Information from patients' charts was transcribed onto Joint Program for the Study of Abortion III forms, which were then keypunched and edited. Details of this study have been published.[10]

Cases of hydatidiform mole were identified in two ways: by pathologic examination of the tissue (35 cases) and by operator report of hydatidiform mole as a complication of abortion (28 cases). Fifteen cases identified by both methods were counted only once.

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Table I. Rates of hydatidiform mole by various characteristics

Characteristic	*No. of cases*	*No. of procedures*	*Cases per 10,000 procedures*	*Risk ratio (95% confidence limits)*
Age (yrs)				
≤14	2	1296	15.4	10.4 (1.5-71.8)*
15-19	22	28229	7.8	5.3 (0.9-31.6)
20-24	22	28940	7.6	5.1 (0.9-31.0)
25-29	11	14329	7.7	5.2 (0.8-32.5)
30-34	1	6767	1.5	Referent group
35-39	1	3219	3.1	2.1 (0.1-31.6)
40-44	1	1136	8.8	6.0 (0.5-68.1)
≥45	2	126	158.7	107.4 (35.7-325.3)*
Race				
White	34	46152	7.4	1.2 (0.3-5.0)
Black	11	14464	7.6	1.2 (0.3-5.6)
Oriental	3	2705	11.1	1.8 (0.3-10.5)
Others	2	3247	6.2	Referent group
Unknown	13	17677	7.4	—
No. of previous pregnancies				
None	36	40279	8.9	1.7 (0.8-3.3)
One	10	18643	5.4	Referent group
Two or more	17	25201	6.8	1.3 (0.6-2.7)
No. of previous spontaneous abortions				
None	57	77202	7.4	1.2 (0.2-8.7)
One	5	5402	9.3	1.5 (0.2-12.8)
Two or more	1	1641	6.1	Referent group
Gestational age at time of procedure				
≤12 weeks	49	62720	7.8	1.2 (0.2-8.7)
≥13 weeks	14	21525	6.5	Referent group

*Significant at $p < 0.05$ level.

Table II. Comparison of estimated incidences in this study to other studies in the United States

Author	*Interval*	*No. of pregnancies*	*No. of cases*	*Rate per 10,000 pregnancies*	χ^2
Present study	1975-1978	84,318	63	7.5	—
Yen and MacMahon[4]	1956-1965	498,293	344	6.9	0.26 ($p > 0.50$)
McCorriston[5]	1951-1965	122,000	94	7.7	0.01 ($p > 0.90$)
Slocumb and Lund[6]	1957-1968	30,982	18	5.8	0.67 ($p > 0.25$)
Westerhout et al.[7]	1950-1964	153,870*	138	9.0	1.27 ($p > 0.25$)
Hayashi et al.[8]	1970-1977	29,501,796	31,965	10.8	8.47† ($p < 0.005$)

*Rate is per 10,000 deliveries. The number of deliveries estimated from the report = $\frac{138 \times 9.00 \times 10{,}000}{0.897}$

†Significant at $p < 0.05$ level.

The incidence per 10,000 procedures (pregnancies) was calculated for the total study population and for the subgroups. Risk ratios and 95% confidence limits were calculated to compare risks within subgroups. A χ^2 test of significance was calculated to compare the rates obtained in this study with rates estimated by other investigators.

Finally, age- and race-adjusted rates for the pregnant population in the United States were estimated by applying our estimated age- and race-specific rates to the total pregnancies for each subgroup for the study period. Total pregnancies were estimated by adding the number of live births, the reported number of ectopic pregnancies, and the reported number of legal abortions. Spontaneous abortions, which are not reported, were excluded from the number of estimated pregnancies.

Results

Of 84,318 women in the Joint Program for the Study of Abortion III study, 63 were identified as having hydatidiform mole, for an incidence of 7.5 cases per 10,000 pregnancies. The incidence varied with age. The risk of having a hydatidiform mole was lowest for

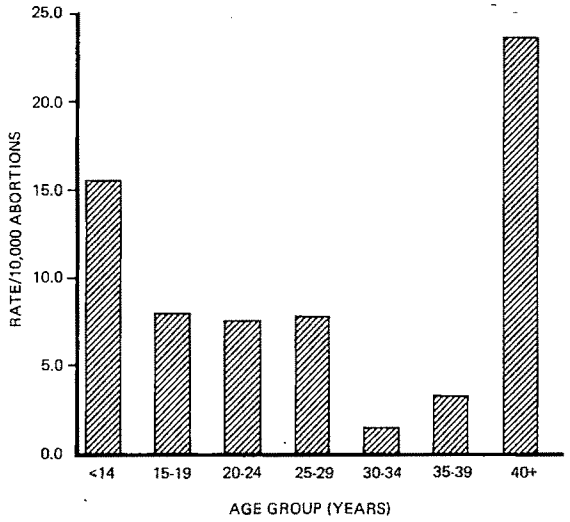


Fig. 1. Hydatidiform mole rate per 10,000 abortions.

women 30 to 34 years of age and highest for women 45 years or older (Table I, Fig. 1). The incidence of hydatidiform mole was highest among Oriental women. However, the difference among the four different racial categories was not significant (Table I).

The age-adjusted rate of 6.9 hydatidiform moles per 10,000 pregnancies and the race-adjusted rate of 7.5 were not significantly different from the crude rate of 7.5 per 10,000 pregnancies.

Women with one or more previous pregnancies had a lower risk of having a hydatidiform mole than women with no previous pregnancies (Table I); this difference was not significant. Women with two or more previous spontaneous abortions had a slightly but not significantly lower risk of having a hydatidiform mole than women who had one or no previous spontaneous abortions (Table I). When stratified by age, the risk did not change significantly with the number of previous pregnancies or the number of previous spontaneous abortions (data not shown).

The rate of hydatidiform mole was higher among abortions done during the first 12 weeks of gestation than among those done at a later gestational age. The rates were not significantly different (Table I).

Our estimated rate was significantly different from the rate estimated by Hayashi et al.,[8] but our estimate was not much different from rates estimated for the United States by other researchers (Table II).

Comment

There are wide variations in the reported incidence of hydatidiform mole from different geographic regions, with higher frequencies generally reported form developing (particularly Oriental) countries. This phenomenon has been explained by some as implying some environmental factor in the etiology of the disease.[7, 11, 12] Within a geographic area, lower socioeconomic status has been an additional factor in some environments[3, 13] but not in others.[4-6, 12] A nutritional factor was hypothesized to explain both the geographic and socioeconomic correlations.[4] The suggestion is that within the United States, even among relatively economically deprived women, the relevant nutritional deficiency is not found.[4] This could explain both the reported lower incidence of hydatidiform mole in the United States and Europe as compared to developing countries and the lack of racial differences found in this and other studies.[6, 11] However, this hypothesis has been tested, and it was repeatedly found that nutrition had no role in the etiology of hydatidiform mole.[4, 6]

Considering race or racial origin to be a separate risk factor associated with hydatidiform mole suggests a hereditary factor. Differences in incidence were reported among populations of different origin in Singapore,[13] Hawaii,[5, 12] California,[7] and Rhode Island.[4] For the United States as a whole, Hayashi et al.[6] found with use of the hospital discharge survey that the incidence of hydatidiform mole among blacks was half that among whites. On the other hand, other studies in Israel[11] and New York[6] reported similar rates for different racial groups. We found that, although the incidence of hydatidiform mole was similar among blacks and whites, it was slightly (but not significantly) elevated for women of Oriental origin.

Probably the only agreed-on risk factor, and the one repeatedly documented as being found, is the increased incidence of hydatidiform mole among teenagers and among women ≥40 years.[4, 7, 8, 11-13] We found a significantly increased incidence for women ≤14 years and for women ≥45 years (Table I, Fig. 1).

Other risk factors for developing hydatidiform mole have been studied, including the number of previous pregnancies, previous spontaneous abortions, and blood groups. However, as is the instance of race, the results are contradictory in most cases.[4, 7] Our study showed no relationship between the incidence of hydatidiform mole and the number of previous pregnancies or the number of previous spontaneous abortions.

In their study of 138 cases of hydatidiform mole in Los Angeles, California, Westerhout et al.[7] noted that, although 75% of all spontaneous abortions would have occurred by the twelfth week of gestation, only about 11% of hydatidiform moles would have terminated by that gestational age. By the twenty-fourth week, 75% of hydatidiform moles would have terminated. Pritchard et al.[2] also report that molar pregnancies terminate from late in the first trimester through the second trimester and that spontaneous expulsion is most likely to occur around the fourth month. Therefore there is some probability that a molar pregnancy will terminate spontaneously before a woman requests a

second-trimester termination procedure. This may be why our reported incidence rates were higher (though not significantly) among abortions done during the first trimester than during the second trimester. This underreporting bias may be an indication that the rate during the first trimester (7.8 per 10,000 pregnancies or 1 per 1280 pregnancies) may be a better estimate of the true rate.

Other factors may have biased our estimate of the incidence of hydatidiform mole. Our study did not eliminate the chance of not counting cases of pregnancies that abort spontaneously at a very early gestational age; we believe, however, that those numbers have been minimized to an extent that they will not significantly affect the numerator (hydatidiform moles) or the denominator (total pregnancies) of our study population.

The number of cases in our study is large enough to estimate with some precision the incidence for the whole population of women obtaining abortions (incidence, 7.5 per 10,000 abortions; 95% confidence limits, 5.8 to 9.6). However, the lack of significant differences among subgroups, particularly when comparing Orientals with women of other races and when comparing women with no previous spontaneous abortions with those with one or more spontaneous abortion, may have resulted from insufficient sample size. The confidence limits around those estimates are quite broad (Table I).

There may be concerns regarding the appropriateness of generalizing our results to the total population of women obtaining abortions in the United States or to the total pregnant population. With respect to the first generalization, the participating clinics, while not a random sample of all clinics, did represent a spectrum of types of facilities and a range of geographic areas.[14] With respect to the second generalization, we know that younger women, older women, and women of races other than white are more likely to have their pregnancies terminated by legal abortion than the rest of the population of pregnant women.[15] Thus we expect those subgroups to be overrepresented in our study group; if one subgroup has a higher risk of having hydatidiform mole (as is the case for younger and older women), our estimated rate may be confounded by that factor. To correct for confounding, we estimated age- and race-adjusted rates. Both adjusted rates were not significantly different from the crude rate.

Ascertainment bias is unlikely to have influenced our estimate. Trained abstractors coded information onto a standard form at the facilities, and an interabstractor reliability study confirmed accurate coding of complications.[10] Misclassification is also unlikely to have occurred, since the diagnosis was made by pathologic examination of the tissue in 35 cases and was based on the operator's report of hydatidiform mole after visual examination of the tissues in the remaining 28 cases.

The pregnant population seeking induced abortion in our study provides an excellent denominator for which a good numerator was obtained by pathologic reports as well as visual examination of tissues. We believe that our study provides a good estimate of the true incidence of hydatidiform mole in early pregnancy.

Assuming that the incidence of hydatidiform mole is 7.5 per 10,000 pregnancies, it is expected that there will be around 3800 women with hydatidiform mole among the approximately 5 million pregnant women in the United States every year.[15, 16] Considering the inconvenience, cost, and risk to which these women are subjected, hydatidiform mole clearly represents a serious public health problem.

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Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women

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Two-dimensional and M-mode echocardiograms were obtained during the thirty-second week of gestation from 69 women classified as follows: group I, 22 normotensive primigravid women; group II, 16 primigravid women with pregnancy-induced hypertension; group III, 21 pregnant women with essential hypertension; and group IV, 10 normotensive nonpregnant control subjects. Systolic, diastolic, and mean arterial pressures were higher in groups II and III than in groups I and IV ($p < 0.001$). Echocardiographic dimensions were significantly increased in group III compared with the other groups ($p < 0.01$). No significant differences were observed among the other groups in the echocardiographic parameters or in the indices of ventricular performance studied. In echocardiographic studies, chronic hypertensive pregnant women are distinguished from patients with pregnancy-induced hypertension because the former have ventricular hypertrophy resulting from the pressure overload exerted for a long period of time. Our patients with essential hypertension experienced no changes in left ventricular performance because of the early stage of their hypertensive disease. (Am J Obstet Gynecol 1986;154:910-3.)

Key words: Heart, hypertension, pregnancy

From a clinical point of view the natural history of hypertension in pregnancy and its mediate prognosis have not been clearly defined yet. The only means of correlating the clinical and histopathologic findings[1, 2] is through renal biopsy performed within 60 days of delivery. However, since this is an invasive method, it should only be used in selected cases.

Two-dimensional and M-mode echocardiography has proved to be a reliable method to study left ventricular dimensions and function,[3, 4] but so far, echocardiographic studies in normotensive and preeclamptic pregnant women have been performed to assess left ventricular function without focusing on the structural features of the heart.[5, 6] In this study we compared the echocardiographic data obtained from normal, preeclamptic and hypertensive pregnant women in order to obtain a clinical index to differentiate previously hypertensive women from patients with pregnancy-induced hypertension.

Material and methods

Three groups of pregnant women and a nonpregnant control group were included in this study (Table I). Group I consisted of 22 normotensive primigravid women aged 21 to 42 years (mean, 23 years) who had no evidence of any cardiorespiratory disease and whose pregnancies were uncomplicated. Group II comprised 16 pregnancy-induced hypertensive primigravid women aged 16 to 36 years (mean, 26 years) who had no previous history of essential hypertension and whose blood pressure returned to normal after delivery. Hypertension was diagnosed when blood pressure was higher than 140 mm Hg systolic and 90 mm Hg diastolic. Group III consisted of 21 women with essential hypertension whose preexisting elevated blood pressure continued throughout pregnancy and puerperium, as confirmed by 60 days' follow-up after delivery. The age range of these women was 18 to 33 years (mean, 29 years). Finally, a control group, group IV, consisting of 10 normotensive nonpregnant women aged 18 to 27 (mean, 25 years) was also studied.

Table I. Classification of the population studied

Group	*Description*	*n*	*Mean age (yr)*
I	Normotensive primigravid	22	23
II	Pregnancy-induced hypertensive	16	26
III	Essential hypertensive	21	29
IV	Normotensive nonpregnant (control group)	10	25

The echocardiographic studies were performed during the thirty-second week of gestation in all pregnant

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Table II. Characteristics of the population studied

	Age (yr)	*Gestational age (wk)*	*Parity*	*Heart rate (bpm)*	*Systolic blood pressure (mm Hg)*	*Diastolic blood pressure (mm Hg)*	*Mean blood pressure (mm Hg)*
Group I—normotensive (n = 22)	23 (21-24)	32	0	98 ± 10	112.18 ± 9	69.73 ± 7	83.98 ± 7
Group II—preeclamptic (n = 16)	26 (16-36)	32	0	102 ± 16	146 ± 11	97.56 ± 7	113.27 ± 9
Group III—essential hypertensive (n = 21)	29 (18-33)	32	0-6	90 ± 13	149.62 ± 13	96.33 ± 9	114.1 ± 9
Group IV—normotensive nonpregnant (n = 10)	25 (19-27)	—	—	74 ± 10	123.3 ± 6	71 ± 6	88.43 ± 4
Group I vs. group II				NS	$p < 0.001$	$p < 0.001$	$p < 0.001$
Group I vs. group III				NS	$p < 0.001$	$p < 0.001$	$p < 0.001$
Group I vs. group IV				$p < 0.01$	NS	NS	NS
Group II vs. group III				NS	NS	NS	NS
Group II vs. group IV				$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Group III vs. group IV				$p < 0.01$	$p < 0.005$	$p < 0.001$	$p < 0.001$

groups. Blood pressure and heart rate were obtained with the patient in the supine position with use of a Dinamap 845 XT monitor and a 1900 Critikon recorder.

Echocardiographic studies. Two-dimensional and M-mode echocardiograms were obtained with a phased array, wide angle Toshiba System TSH-10A with a 2.25 MHz transducer focused at 7.5 cm, with the patient in either the supine or lateral position. The transducer was placed in the third or fourth intercostal space at the left parasternal border. Measurements were made according to standard techniques.[7, 8] The 11 echocardiographic parameters studied were as follows: end diastolic dimension of left ventricle, end systolic dimension of left ventricle, end diastolic dimension of right ventricle, diastolic interventricular septum, diastolic posterior wall thickness of left ventricle, left atrium, aorta, left ventricular mass, end systolic stress, left ventricular dimension shortening, and end systolic stress/end systolic volume index.

The technique of Devareux and Reichek[9] was used to measure left ventricular mass (LVM):

$$LVM = 1.04\,[(EDDp + DIVSp + DPWTp)^3 - (EDDp)^3] - 13.6$$

where p indicates modified (Penn) convention for determining EDD (end-diastolic dimension of the left ventricle), DIVS (diastolic interventricular septum), and DPWT (diastolic posterior wall thickness of the left ventricle).

To determine end-systolic stress (ESS), the method of Wilson et al.[10] was used:

$$ESS = \frac{0.334 \times SBP \times ESD}{SPWT \times (1 + SPWT/ESD)}$$

where ESD = end-systolic diameter of the left ventricle, SBP = systolic blood pressure when echocardiograms were recorded, and SPWT = systolic posterior wall thickness of the left ventricle.

Percentage fractional shortening (PFS) was determined as follows[11, 12]:

$$PFS = \frac{EDD - ESD}{EDD} \times 100$$

and PFS and ESS/ESVI (end-systolic stress/end-systolic volume index ratio) were used as the ventricular index.[13] The method of Teicholz et al.[14] was used to determine end-systolic volume (ESV).

Statistical study. All data are expressed as mean ± standard deviation. Statistical significance was calculated by the analysis of variance.

Results

Systolic, diastolic, and mean blood pressures were significantly higher in patients with preeclampsia and essential hypertension than in the other groups ($p < 0.001$). However, no differences were found between the essential hypertensive and preeclamptic patients. Preeclamptic, normotensive, and essential hypertensive pregnant patients showed a higher heart rate than the nonpregnant control women (Table II).

Echocardiographic measurements. When compared with the other groups, patients with essential hypertension had a significant increase in EDD, DIVS, DPWT, and LVM. With regard to aorta dimensions, only a slight difference was observed between essential hypertensive and normotensive pregnant women. No significant differences were observed among the normotensive, preeclamptic, and control women, with the exception of left ventricular mass, which was greater in the preeclamptic women than in the control group.

In contrast with these observations, the ESD of the right ventricle, as well as ESS were similar in all groups.

Table III. Results of the study

	End diastolic dimension of left ventricle (mm)	*End systolic dimension of left ventricle (mm)*	*End diastolic dimension of right ventricle (mm)*	*Diastolic interventricular septum (mm)*
Group I—normotensive (n = 22)	46.9 ± 4	29.23 ± 5	16.0 ± 6	8.64 ± 1
Group II—preeclamptic (n = 16)	47.31 ± 3	30 ± 4	17.1 ± 5	9 ± 1
Group III—essential hypertensive (n = 21)	49.81 ± 4	30.75 ± 4	17.4 ± 4	9.95 ± 2
Group IV—normotensive nonpregnant (n = 10)	45.6 ± 2	28.9 ± 2	16.6 ± 2	7.1 ± 1
Group I vs. group II	NS	NS	NS	NS
Group I vs. group III	$p < 0.01$	NS	NS	$p < 0.01$
Group I vs. group IV	NS	NS	NS	NS
Group II vs. group III	$p < 0.05$	NS	NS	$p < 0.05$
Group II vs. group IV	NS	NS	NS	NS
Group III vs. group IV	$p < 0.05$	NS	NS	$p < 0.01$

LVM increased significantly in essential hypertensive women, compared with the other groups. Left PFS and the ESS/ESV ratio index, as an expression of ventricular systolic performance, were normal in the groups with preeclampsia and essential hypertension; furthermore, no significant differences were found among the groups (Table III). There was no echocardiographic evidence of left ventricular diastolic dysfunction in any of the groups.

Comment

The physiologic changes observed during pregnancy, such as the increase in maternal metabolic requirements, the decrease in peripheral vascular resistance and the increase in blood volume, cause significant changes in cardiac output during pregnancy as early as the thirteenth week of gestation,[15, 16] as evidenced by the increase in cardiac output and systolic volume.[15, 16]

Rubler et al.[15] performed echocardiographic studies in normotensive patients. These results showed a progressive increase in left and right ventricular dimensions from the thirteenth week of gestation on. These observations demonstrate that these physiologic changes appear concomitantly with the necessary increase in cardiac output to compensate for the augmented metabolic requirements during gestation. The echocardiographic data obtained from our population of normotensive pregnant women at the thirty-second week of gestation are in agreement with the findings reported by Rubler et al.[15] The results obtained by other investigators on the left ventricular systolic function in patients with pregnancy-induced hypertension vary widely; Kuzniar et al.[6] found a reduced cardiac output and ESV in preeclampsia. However, no differences between normotensive and hypertensive women were found by Assali et al.[17] We found that at the thirty-second week of gestation, ventricular systolic performance was normal in all the groups studied. This difference in results among some papers may be due to the methods used and/or the selection of the population. In our study echocardiograms have shown significant differences in cardiac structures. Thus essential hypertensive patients showed a significant increase in left ventricular PWT, interventricular septum, LVM, and EDD of the left ventricle. In contrast, left ventricular dimensions remained unchanged in preeclampsia, since hypertension only lasts the course of gestation and blood pressure may not be excessively high, as found in this study. In essential hypertension, left ventricular hypertrophy as well as a slight increase in EDD of the left ventricle occur in order to preserve left ventricular systolic function and compensate for the pressure effect and volume load of the heart. Only pregnant women with essential hypertension showed echocardiographic structural differences, although ventricular systolic function was normal, which may indicate that these patients are in an early stage of the development of hypertension.

Our study showed that echocardiographic changes were only observed in pregnant women with essential hypertension. Thus we propose the use of echocardiography as another parameter in the differentiation of pregnancy-induced hypertension from essential hypertension in pregnant women.

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Diastolic posterior wall thickness of left ventricle (mm)	*Left atrium (mm)*	*Aorta (mm)*	*Left ventricular mass (g)*	*End systolic stress*	*Left ventricular dimension shortening (%)*	*End systolic stress/end systolic volume*
8.27 ± 1	30.8 ± 6	25 ± 3	154.65 ± 31	45.8 ± 13	38.27 ± 8	3.01 ± 0.84
9 ± 1	33.3 ± 6	27.0 ± 2	170.36 ± 47	50.12 ± 13	38.19 ± 7	3.06 ± 0.94
9.6 ± 2	36.4 ± 5	28.5 ± 3	213.84 ± 66	51.74 ± 12	36.75 ± 4	3.09 ± 0.84
7.7 ± 1	32.8 ± 2	29.9 ± 1	117.65 ± 18	51 ± 3	35.70 ± 6	3.08 ± 0.18
NS	NS	NS	NS	NS	NS	NS
$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$	NS	NS	NS
NS	NS	NS	NS	NS	NS	NS
$p < 0.05$	NS	NS	$p < 0.005$	NS	NS	NS
NS	NS	NS	$p < 0.05$	NS	NS	NS
$P < 0.01$	$P < 0.01$	NS	$P < 0.001$	NS	NS	NS

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Sodium excretion in human pregnancy: A role for arginine vasopressin

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Plasma arginine vasopressin concentration was measured before and after 1 week's dietary sodium loading (>250 mmol/day) or deprivation (<50 mmol/day) in 50 second-trimester and 34 third-trimester primigravidas. A significant rise in plasma arginine vasopressin was observed only in second-trimester subjects following sodium loading (5.6 ± 0.5 to 6.6 ± 0.5 pg/ml; $p < 0.05$). Plasma sodium concentration and blood pressure did not alter, and plasma volume increased slightly in this group (24.4 ± 0.5 to 25.9 ± 0.7 ml/cm height; $p < 0.01$). Hence the observed increment in plasma arginine vasopressin concentration in this group could not be explained by alterations to the major factors regulating arginine vasopressin release. It is suggested that arginine vasopressin secretion increases to assist natriuresis following dietary sodium loading in the second trimester of human pregnancy. (Am J Obstet Gynecol 1986;154:914-9.)

Key words: Arginine vasopressin, natriuresis, plasma volume

The regulation of sodium excretion in human pregnancy remains poorly understood. Although there is a net sodium retention of approximately 3 mmol/day,[1] the mechanisms determining this final sodium balance are unclear. Several hormones, including aldosterone, deoxycorticosterone acetate, and progesterone, are known to play a considerable role in the control of sodium excretion, but the role of other hormones is less clear.

Although a role has been described for arginine vasopressin in sodium regulation in man and other mammals, the effects of arginine vasopressin on sodium excretion in pregnancy are uncertain. The only two studies that have addressed this question in human pregnancy have produced conflicting results, one noting a considerable sodium-retaining effect[2] and the other demonstrating a marked natriuretic effect of this hormone.[3] A sodium-retaining action of this hormone might contribute to the salt and water retention that is characteristic of normal pregnancy and enhanced in many cases of established pregnancy-associated hypertension (preeclampsia).[4]

Conversely, if the predominant effect of arginine vasopressin on sodium is to enhance its excretion during pregnancy, then the result might be relative contraction of the plasma volume from its normally expanded state, which is characteristic of pregnancy-associated hypertension.[5]

Understanding the effects of arginine vasopressin on sodium excretion in humans has been complicated by the need to extrapolate data from animal studies or from studies utilizing exogenous infusions of arginine vasopressin. The latter pose the particular difficulty of relating the effects of arginine vasopressin infusions to actual physiologic events. However, the development of sensitive and specific radioimmunoassays for the measurement of plasma arginine vasopressin has already provided new concepts of the physiology of this hormone in human pregnancy[6] and might provide insights into its proposed role in regulating sodium excretion.

If arginine vasopressin is indeed a sodium-regulatory hormone in human pregnancy, then it could be expected that endogenous plasma arginine vasopressin concentrations alter in response to changes in dietary sodium intake to restore sodium balance. However, other factors, such as plasma osmolality, blood pressure, and plasma volume, are the major known determinants of arginine vasopressin secretion,[7] and these factors might also be influenced by an alteration in sodium intake.

Therefore we monitored these factors together with plasma arginine vasopressin concentrations before and after dietary sodium loading or deprivation during the second and third trimesters of human pregnancy in order to seek evidence for a role for this hormone in regulating sodium excretion.

Material and methods

Clinical studies. Eighty-four studies were made in 58 primigravidas who remained normotensive through-

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Table I. Subject characteristics at each study period

	Second trimester		*Third trimester*	
	High-salt diet	*Low-salt diet*	*High-salt diet*	*Low-salt diet*
No. of subjects	23	27	16	18
Age (yr)	28.1 ± 1.1	28.3 ± 0.8	28.7 ± 1.1	28.5 ± 1.0
Height (cm)	166.5 ± 1.7	164.7 ± 1.1	166.4 ± 2.0	165.2 ± 1.6
Gestation (wk)	23.9 ± 0.4	23.8 ± 0.3	33.9 ± 0.3	33.7 ± 0.3

out their pregnancy. All were participants in a large prospective study of sodium control in pregnancy and had no prior history of hypertension or of renal or cardiac disease. After giving written informed consent, subjects were assigned randomly to either a high-salt or low-salt diet, which was taken for 1 week during the second trimester (22 to 26 weeks' amenorrhea) or third trimester (32 to 36 weeks' amenorrhea). Thirteen subjects in each dietary group had studies during both trimesters.

The high-salt diet consisted of a food intake with approximately 150 mmol of sodium/day supplemented by sodium chloride capsules (17 mmol sodium/capsule) to a total intake of approximately 300 mmol/day, the dietary aim being a sodium intake of ≥250 mmol/day. Those subjects undertaking a low-salt diet were instructed in a dietary sodium intake of approximately 20 mmol/day, the dietary aim being a sodium intake of <50 mmol/day. All subjects collected urine for 48 hours before the commencement of their diet and for the last 48 hours of their diet, for measurement of urine volume and of sodium and creatinine excretion. At the commencement (day 0) and completion (day 7) of the diet, subjects attended a special clinic between 7:00 and 10:00 AM, where blood was collected for the measurement of plasma sodium concentration, plasma arginine vasopressin concentration, plasma renin activity, and plasma aldosterone concentration, and plasma volume was measured. On these same days the blood pressure was measured after 10 minutes' rest, with the patient in the sitting position. Diastolic blood pressure was recorded at the phase IV Korotkoff sound with the use of a Hawkesly random zero sphygmomanometer.

Analytical methods

Collection and handling procedures. For measurement of plasma arginine vasopressin concentration, 10 ml of blood was placed immediately into tubes that had been prechilled in ice and that contained disodium ethylenediaminetetra-acetic acid (Na_2 EDTA). After centrifugation in the cold, plasma was separated and extracted within 3 hours. Five milliliters of blood was also drawn into a tube containing Na_2 EDTA and handled at room temperature before snap freezing for later determination of plasma renin activity and plasma aldosterone concentration. A further 20 ml of blood was collected for measurement of the biochemical profile, including plasma sodium concentration, and as a "blank" sample for determination of the plasma volume.

Laboratory methods. Plasma arginine vasopressin was measured by a modification of the method of Woods.[8] The addition of phenanthroline monohydrate caused significant displacement of the standard curve and prevented its use as an inhibitor of the vasopressinases. Cold and Na_2 EDTA were used as inhibitors, and the extraction was performed in ice slush with Na_2 EDTA added to the buffer. Plasma was extracted with the use of acetone and petroleum ether, and recovery of added synthetic arginine vasopressin was consistently 65% to 70% in nonpregnant subjects and in both second- and third-trimester subjects. Standard curves were prepared in duplicate over a range of 0.5 to 125 pg arginine vasopressin per tube by means of the international standard for arginine vasopressin.* Iodinated arginine vasopressin,† with a specific activity of 2200 μCi/μmol, was incubated with standard arginine vasopressin and with arginine vasopressin antibody for 24 hours at room temperature. The antibody, prepared in rabbits, has a 10% cross-reactivity with lysine vasopressin and less than 1% cross-reactivity with oxytocin. Separation of bound and free arginine vasopressin was achieved with a solid-phase second antibody-coated cellulose suspension (Sac-cel),‡ and bound arginine vasopressin radioactivity was counted by gamma counting for 4 minutes per tube. Results were not corrected for recovery and are expressed as picograms of arginine vasopressin per milliliter of plasma.

Assay sensitivity was 1.3 pg/ml, the intra-assay coefficient of variation was 9.4 ± 0.9% (n = 20 assays), and the interassay coefficient of variation was 13.4 ± 1.0% (n = 9 assays). The 50% displacement value was 7.9 pg/ml. Under conditions of normal hydration and eating without restriction, the mean plasma arginine vasopressin concentration was 5.1 ± 0.4 pg/ml in 20 healthy nonpregnant volunteers and 5.1 ± 0.2 pg/ml in 49 primigravid pregnant women studied at a mean gestation of 29 ± 0.4 weeks.

Plasma renin activity was measured by radioimmu-

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†Dupont, Boston, Massachusetts.
‡Wellcome, Beckenham, England.

Table II. Urine sodium excretion before and after dietary manipulations

	Second trimester		*Third trimester*	
	High-salt diet	*Low-salt diet*	*High-salt diet*	*Low-salt diet*
Urine sodium excretion				
Day 0	111 ± 9	109 ± 11	89 ± 8	97 ± 8
Day 7	240 ± 17*	27 ± 3*	242 ± 24*	27 ± 3*
Urine Na:Cr ratios				
Day 0	12 ± 1	11 ± 1	11 ± 1	11 ± 1
Day 7	28 ± 2*	3 ± 1*	27 ± 2*	3 ± 1*

*$p < 0.001$, day 0 vs. day 7.

noassay of angiotensin I with care to avoid cryoactivation.[9] Plasma aldosterone concentration was measured by radioimmunoassay, plasma sodium concentration by the AutoAnalyzer method, and plasma volume by an Evans blue dye–dilution technique as previously described.[5]

Urine sodium was measured by flame photometry,* and urine creatinine was determined spectrophotometrically after colorimetric reaction with alkaline picrate. Results for urine sodium excretion were then expressed both as millimoles per day and as millimoles of sodium excreted per millimole of creatinine excreted (Na:Cr ratio) from the 2-day urine collections, the latter method being used as a check for adequacy of urine collections.

Statistical analysis. Within-patient differences were analyzed by paired Student's *t* test and differences between groups by unpaired Student's *t* test. Correlations were evaluated by determination of the Pearson correlation coefficient. Results are expressed as the mean ± SEM.

Results

Subjects. During the second trimester, studies were made in 23 subjects in the high-salt group and 27 subjects in the low-salt group (Table I). In the third trimester the same studies were made in 16 subjects undertaking a high-salt diet and in 18 subjects using a low-salt diet. Thirteen subjects in each dietary group had studies made during both trimesters. Results in these subjects did not differ from those in the larger groups from which they arose. There was no significant difference between dietary groups in age, height, or stage of gestation.

Urinary sodium excretion. There was no significant difference in baseline (day 0) urinary sodium excretion or urine Na:Cr ratio between dietary groups in the second or third trimesters (Table II). The urinary sodium excretion rose significantly ($p < 0.001$) following a high-salt diet and fell significantly ($p < 0.001$) following a low-salt diet in both trimesters. The same pattern of changes was observed for urine Na:Cr ratios.

Plasma arginine vasopressin concentrations. Plasma arginine vasopressin concentration rose significantly ($p < 0.05$) following a high-salt diet in second-trimester subjects but not in third-trimester subjects eating this same diet (Fig. 1). No significant change in plasma arginine vasopressin concentration was observed after a low-salt diet in either trimester.

Factors regulating plasma arginine vasopressin concentration and sodium excretion. Plasma sodium concentration was altered only in the low-salt group during the second trimester (Table III). This change was accompanied by a small but significant fall in the plasma volume (0.9 ml/cm; $p < 0.05$). The plasma volume increased slightly but significantly in the high-salt groups during both trimesters and remained constant in the low-salt group during the third trimester.

Blood pressure was not significantly influenced by any dietary manipulation, but plasma renin activity and plasma aldosterone concentration rose significantly in both low-salt groups and fell significantly in both high-salt groups.

Data were further analyzed within the second-trimester high-salt group, where an increase in mean plasma arginine vasopressin concentration was observed. Changes in plasma arginine vasopressin concentrations for each subject were analyzed with the corresponding changes in plasma renin activity, urine sodium excretion, and diastolic blood pressure, but no significant correlations between arginine vasopressin concentrations and these parameters were observed.

Comment

We have found an increase in plasma arginine vasopressin concentration in response to dietary sodium loading in the second trimester of human pregnancy. No change in blood pressure or plasma sodium concentration occurred with this maneuver, and the increment in plasma volume should, if anything, have resulted in a marginal fall in arginine vasopressin secretion.[10] Therefore some other factor must have provided

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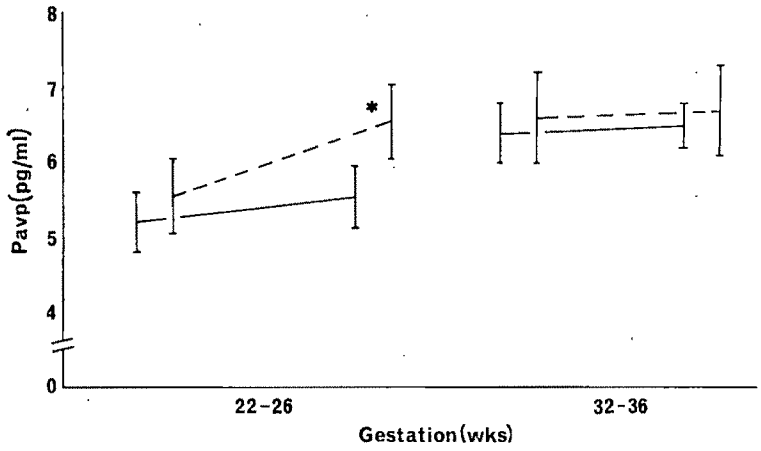


Fig. 1. Plasma arginine vasopressin *(Pavp)* levels before and after dietary sodium manipulations. *Dashed line,* High-salt diet; *solid line,* low-salt diet; * $p < 0.05$, day 0 versus day 7.

Table III. Factors regulating arginine vasopressin release and sodium excretion

	Second trimester		*Third trimester*	
	High-salt diet	*Low-salt diet*	*High-salt diet*	*Low-salt diet*
Plasma sodium (mmol/L)				
Day 0	137 ± 0.3	138 ± 0.3	137 ± 0.5	137 ± 0.4
Day 7	137 ± 0.3	137 ± 0.4*	137 ± 0.5	137 ± 0.5
Plasma volume (ml/cm)				
Day 0	24.4 ± 0.5	24.1 ± 0.5	26.4 ± 0.8	26.0 ± 0.7
Day 7	25.9 ± 0.7†	23.2 ± 0.5*	27.3 ± 0.7*	25.4 ± 0.6
SBP (mm Hg)				
Day 0	103 ± 2	101 ± 2	103 ± 2	104 ± 3
Day 7	103 ± 3	99 ± 2	107 ± 2	107 ± 2
DBP (mm Hg)				
Day 0	63 ± 2	60 ± 2	64 ± 2	69 ± 2
Day 7	64 ± 2	60 ± 2	69 ± 2	69 ± 2
PRA (ng AI/ml/hr)				
Day 0	4.0 ± 0.6	3.7 ± 0.4	4.9 ± 0.7	6.7 ± 0.7
Day 7	2.5 ± 0.3†	8.5 ± 1.0‡	2.6 ± 0.5‡	12.0 ± 1.5‡
PAC (fmol/ml)				
Day 0	1527 ± 274	1574 ± 168	2041 ± 230	2896 ± 421
Day 7	1103 ± 135*	2637 ± 272‡	1376 ± 202‡	3599 ± 365*
PAVP (pg/ml)				
Day 0	5.6 ± 0.5	5.2 ± 0.4	6.6 ± 0.6	5.9 ± 0.4
Day 7	6.6 ± 0.5*	5.6 ± 0.4	6.7 ± 0.6	6.0 ± 0.3

SBP = systolic blood pressure; DBP = diastolic blood pressure; PRA = plasma renin activity; AI = angiotensin I; PAC = plasma aldosterone concentration; PAVP = plasma arginine vasopressin.

*Day 0 versus day 7, $p < 0.05$.

†Day 0 versus day 7, $p < 0.01$.

‡Day 0 versus day 7, $p < 0.001$.

the stimulus for the observed increase in plasma arginine vasopressin concentration in this group.

The major determinants of arginine vasopressin release have been reviewed by Robertson.[10] In addition to osmotic, volume, and blood pressure stimuli, only nausea appears to be capable of significantly influencing arginine vasopressin release in nonpregnant subjects. Further, changes in plasma sodium concentration appear necessary to exert any osmotic influence on arginine vasopressin release, and infusions of glucose, although increasing plasma osmolality, do not increase arginine vasopressin secretion.[11] None of our patients complained of nausea at the time of their study, and the plasma sodium concentration did not rise significantly in any subject. Other stimuli, such as angiotensin II and hypoglycemia, appear to be very weak in their ability to elicit arginine vasopressin release and are not thought to be of clinical importance in disorders of water balance.[10] Plasma renin activity, and presumably then angiotensin II, fell in our high-salt group, which

demonstrated a rise in plasma arginine vasopressin concentration, and none of these subjects developed hypoglycemia. Thus the major known stimuli for the release of arginine vasopressin cannot explain the increment in this hormone that we observed to be a response to sodium loading in the second trimester. The observed rise in endogenous plasma arginine vasopressin concentration may therefore represent an attempt to enhance renal sodium excretion by this hormone, as suggested previously for pregnant women.[3] This possibility is supported by the finding that plasma arginine vasopressin concentration rises significantly in at least one other group, nonpregnant patients with essential hypertension, following a 250 mmol/day sodium intake.[12] Although significant changes in the plasma volume were observed following alterations in sodium intake in this study, these changes were small. This constancy of plasma volume has no doubt been mediated partly by appropriate changes in the renin-aldosterone system, but it is also possible that arginine vasopressin assisted the natriuresis following sodium loading in the second trimester. However, the understanding of arginine vasopressin in relation to sodium excretion is limited. Some studies have demonstrated natriuretic effects of this hormone,[13] although the infusion doses of arginine vasopressin have sometimes been sufficiently high to elicit a pressor response and thereby secondarily promote natriuresis. Similarly, overhydration per se is capable of promoting natriuresis, and many studies, in an attempt to suppress endogenous arginine vasopressin production, have utilized this procedure. Recently, Field et al.[14] studied the effects of arginine vasopressin infusions in Brattleboro rats (who produced no endogenous arginine vasopressin) and were unable to demonstrate any role for arginine vasopressin in regulating sodium excretion. Conversely, others have claimed that arginine vasopressin promotes sodium retention perhaps in the ascending limb of Henle's loop.[15] No similar studies appear to have been performed in pregnant animals.

In man, arginine vasopressin is thought to promote fractional sodium reabsorption, urea reabsorption, and potassium excretion.[16] In two studies of the effects of arginine vasopressin in human pregnancy, sodium excretion has been found to fall and increase. In the former study,[2] large doses of Pitressin were administered, and the consequent reduction in glomerular filtration rate was the most likely explanation for the decreased urine sodium excretion. Calculation of the fractional sodium excretion in these subjects shows that tubular sodium reabsorption was variably altered, in some cases increasing. In contrast, Torres el al.[3] infused very small doses of arginine vasopressin and found a doubling of the fractional sodium excretion in second-trimester subjects but only a minimal increase in third-trimester subjects. Our data regarding endogenous arginine vasopressin concentrations are consistent with these latter findings and suggest a natriuretic effect of arginine vasopressin, operative in second-trimester but not third-trimester pregnant women following dietary sodium loading. Recent evidence suggests that a fall in plasma arginine vasopressin concentration following dietary sodium deprivation would not be expected, since arginine vasopressin appears important in the regulation of sodium excretion only when the body is loaded with sodium.[17] This finding is supported by the absence of a fall in plasma arginine vasopressin concentration after a low sodium intake by the pregnant women studied here. Although arginine vasopressin may enhance sodium excretion, we have shown that it is not regulated by the body's sodium status in a manner similar to other primary sodium-regulating hormones such as aldosterone, which we confirmed to rise and fall appropriately in our subjects following dietary sodium manipulation.

In attempting to explain the mechanism for arginine vasopressin–assisted natriuresis, we examined the relationship between changes in arginine vasopressin concentration and changes in plasma renin activity in second-trimester sodium-loaded subjects, since arginine vasopressin is capable of suppressing renin release.[18] No significant correlation between these two parameters was observed in this study, making it unlikely that the natriuretic effect of arginine vasopressin is mediated by renin suppression.

This study is the first to monitor endogenous plasma arginine vasopressin levels in human pregnant subjects in response to dietary sodium loading. The findings support previous suggestions[3] that arginine vasopressin plays a role in sodium excretion in the second trimester of pregnancy. If this hormone is involved in the pathogenesis of the hypertensive disorders of pregnancy, then its actions might explain both the contracted plasma volume and the increased total body water that may accompany these disorders. Further examination of the role of arginine vasopressin and its relationship to sodium excretion and water balance in both pregnancy-associated hypertension and chronic hypertension in pregnancy will elucidate its significance in their pathogenesis.

We thank the obstetricians of the Royal North Shore Hospital, Sydney, for allowing us to study their patients, Prof. A.Z. Gyory for advice and discussion, and Sister Margaret Ross, Dr. Georgina Crawford, Mrs. Ellen Nicholson, and Miss Helen Norton for their expert technical assistance.

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Profound hypotension complicating magnesium therapy

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Two preeclamptic women became profoundly hypotensive while receiving magnesium sulfate. No evidence of toxic levels was present in either. In both, preeclampsia-induced hypovolemia was extreme. That nontoxic magnesium levels can cause severe hypotension is an alarming possibility. Acknowledgment of this complication and expeditious reversal of the problem is essential. (AM J OBSTET GYNECOL 1986;154:919-20.)

Key words: Preeclampsia, magnesium sulfate, hypotension

Magnesium sulfate administered at accepted therapeutic doses for preeclampsia influences blood pressure slightly.[1] Significant hypotension is not a generally recognized side effect. Two preeclamptic women treated with a well-established dosage schedule[2] designed to produce serum magnesium levels of 6 to 8 mEq/L became severely hypotensive. They did not have toxic magnesium levels, and their response to the drug is in contrast to a large number of preeclamptic patients receiving the drug according to the same protocol.

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Case reports

Case 1. A primigravid woman presented to an outlying hospital at 35 weeks' gestation with edema, proteinuria, and blood pressure of 180/120 mm Hg. After 3 hours of intravenous magnesium sulfate administration (total dose of 6 gm), the blood pressure dropped to 64/40 mm Hg. Magnesium was discontinued, and blood pressure rose to 160/98 mm Hg within 15 minutes. Pulse remained 70 bpm throughout.

After transfer the blood pressure was 210/136 mm Hg and pulse was 104 bpm. Laboratory studies were normal except for serum albumin level of 2.5 gm/dl.

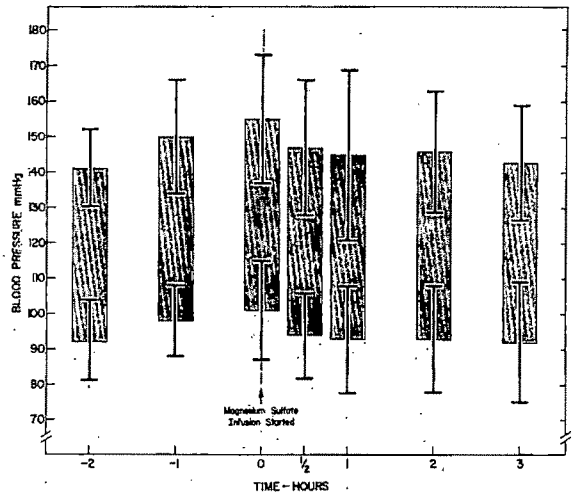


Fig. 1. Systolic, diastolic, and mean arterial pressure changes in 320 preeclamptic women treated with magnesium sulfate.

Serum calcium level was 7.6 mg/dl and magnesium level was 4.1 mEq/L.

Magnesium sulfate was administered intravenously by calibrated infusion pump at a rate calculated to deliver 8400 mg in the first hour. Within 20 minutes blood pressure was 70/50 mm Hg, and the infusion was discontinued. Blood pressure returned promptly to hypertensive levels. Serum magnesium level drawn minutes after the hypotensive episode was 4.5 mEq/L. The patient then received 1500 ml of intravenous normal saline solution, raising the central venous pressure from 0 to 7. A healthy 1990 gm infant was delivered and the patient became normotensive after delivering.

Case 2. A woman, gravida 2, para 1, with twin gestation, presented to another hospital at 31 weeks with headache, epigastric pain, blood pressure of 160/110 mm Hg, and proteinuria. Before transfer, magnesium sulfate, 10 gm, was given intramuscularly.

On arrival, blood pressure was 178/100 mm Hg and deep tendon reflexes were 3+. Laboratory studies were normal except for albumin of 3.0 gm/dl. Serum magnesium was not measured; calcium level was 8.2 mg/dl. Magnesium sulfate was infused as described previously. This was begun 2 hours after the single intramuscular dose mentioned. Within 15 minutes during which 2100 mg of magnesium sulfate was administered by calibrated pump, blood pressure was 70/50 mm Hg. The infusion was discontinued, and blood pressure rose to 150/94 mm Hg in <10 minutes. Urine output decreased to 25 ml/hour, and central venous pressure was measured at 2. Plasma protein was given slowly, raising the central venous pressure to 7.

Subsequently the patient delivered healthy 2110 and 1410 gm infants. Hypertension resolved slowly after delivery.

Both women received 25 mg of intravenous promethazine along with the magnesium infusion, but neither received any other medication. Both patients were placed in semirecumbent position. Neither had depressed respirations or deep tendon reflexes.

Blood pressure response to magnesium is usually slight in eclamptic patients.[1] An illustration of the blood pressure changes in 320 preeclamptic women given magnesium sulfate infusion according to the regimen of Hall[2] (Fig. 1) demonstrates only a modest mean decrease. In 26 years, with use of this protocol for magnesium administration, no similar instances of hypotension have been recognized. There is no evidence of toxic magnesium levels in these patients, and other women receiving both magnesium and promethazine demonstrated no such blood pressure change. The severe degree of hypovolemia probably combined with magnesium-induced vasodilatation to produce this condition.

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Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia

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One hundred twelve patients in preterm labor were followed prospectively, with electrocardiograms taken before ritodrine therapy and at 6 and 24 hours of treatment. Ninety-six percent of patients developed sinus tachycardia. Other changes were seen in 79% of the study group. These changes included ST segment depression in 70%, T wave flattening or inversion in 55%, and prolongation of the QT interval in 35% of our sample. None of the electrocardiograms showed the presence of a significant axis deviation, a change in QRS interval, or arrhythmia. No correlation was seen between symptoms of ischemia and electrocardiographic changes. A drop in potassium concentration was noted initially, but a direct correlation between potassium concentrations and frequency of electrocardiographic changes was not present. We conclude that the electrocardiographic changes that are often observed during myocardial ischemia may be frequent in asymptomatic patients treated with ritodrine and that these changes may be a physiologic expression of ritodrine-induced tachycardia or hypokalemia. The validity of the use of the presence of electrocardiographic changes as the only criterion for discontinuation of ritodrine therapy is questioned. (AM J OBSTET GYNECOL 1986;154:521-3.)

Key words: Electrocardiography, ritodrine

Since the approval by the Food and Drug Administration of ritodrine as a tocolytic agent, widespread usage has evolved. Past experience with β-adrenergic tocolytics has shown the occurrence of severe cardiovascular complications including pulmonary edema, myocardial ischemia, and myocardial infarction in up to 5% of patients treated parenterally.[1-3] In addition, minor side effects such as nausea, vomiting, palpitations, nervousness, and tremulousness occur at frequencies of up to 30%.[3] Following these reports, most practitioners perform electrocardiographic studies in the presence of chest discomfort or shortness of breath, although some perform these tests routinely. Based on electrocardiographic changes consistent with myocardial ischemia, therapy is often discontinued.[4] However, since the frequency of electrocardiographic changes in asymptomatic patients undergoing ritodrine tocolysis has not been previously established, their specificity as well as relationship to myocardial stress are not clear. Therefore the clinical value of electrocardiograms in the management of these patients has remained unknown.

The following report represents an analysis of electrocardiographic changes associated with ritodrine therapy. Our goal was to identify and characterize these changes during tocolysis and to ascertain whether their presence could be correlated with significant clinical symptoms.

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Materials and methods

One hundred twelve consecutive patients diagnosed as having preterm labor between 26 and 34 weeks' gestation underwent electrocardiography before initiation of tocolysis. The diagnosis of preterm labor was made in the presence of regular uterine contractions leading to cervical change. All patients had normal intake electrocardiograms and no cardiovascular risk factors such as hypertension, cardiac arrhythmias, or angina in their medical histories. The electrocardiograms were repeated at 6 hours of therapy in all patients and again at 24 hours in those patients still receiving intravenous ritodrine therapy. Our ritodrine infusion protocol required meticulous monitoring of fluid administration and vital signs. Fluid intake was limited to 1.8 L/24 hr, since previous reports have shown fluid overload to be the critical factor in development of pulmonary edema.[1, 3, 5] Our ritodrine infusion protocol is as follows:

1. 300 mg of ritodrine per 500 ml of normal saline solution = 600 μg ritodrine per milliliter.
2. Initial ritodrine infusion rate: 100 μg/minute; increase dose 50 μg/minute every 10 to 15 minutes.
3. Monitor vital signs every 10 to 15 minutes.
4. Avoid the following: pulse >120 bpm, systolic pressure <90 torr, diastolic pressure <40 torr.

Table I. Serum potassium ion concentrations after 6 hours of ritodrine infusion in relationship to the frequencies of electrocardiographic abnormalities*

Potassium concentration at 6 hours (mEq/L)	*Normal (%)*	*ST (%)*	*T wave (%)*	*QTc (%)*
>3.5	7	22	13	7
3.0-3.5	13	26	18	7
2.5-2.9	11	7	13	0
<2.5	9	7	0	7

*All patients had initial potassium concentration >3.5 mEq/L.

5. Limit total fluid intake to ≤75 ml/hr.

6. Obtain electrocardiogram, serum electrolytes test, and complete blood count before therapy and at 6 hours.

The electrocardiograms were reviewed by a cardiology specialist. The variables that were evaluated in each electrocardiogram were heart rate (sinus tachycardia = >100 bpm), rhythm, presence of ST depression (>1 mm), T wave inversion or flattening, QRS intervals, axis shifts, and QT intervals.

Results

Two of the 112 patients treated with ritodrine developed serious cardiovascular symptoms during the course of therapy. Both patients experienced significant angina, and one ultimately developed pulmonary edema attributed to a moderate fluid overload, received through excessive intravenous therapy despite a protocol setting rigid limitations for fluid administration. Neither of these patients had an abnormal electrocardiogram.

Ninety-six percent of the patients developed sinus tachycardia during ritodrine therapy. Other electrocardiographic changes occurred in 79% of the study group. These changes included ST segment depression in 70%, T wave flattening or inversion in 55%, and prolongation of the QT interval, corrected for rate, in 35% of our sample. None of the electrocardiograms showed a significant axis deviation, a change in QRS interval, or arrhythmia.

Seventy-six percent of the patients had either ST segment changes, T wave changes, or both. These changes were primarily seen in the inferolateral leads—II, III, AVF, and V3 to V6—the area primarily supplied by the right coronary artery.

The electrocardiographic changes after 24 hours of intravenous therapy demonstrated a persistent, though less marked, sinus tachycardia. Eighty-nine percent of these electrocardiograms showed a return trend toward the baseline pretreatment electrocardiograms. There was a noted resolution of ST-T wave changes toward an isoelectric baseline.

No statistical correlation could be drawn between the rate of ritodrine administration and the incidence of electrocardiographic changes in our population. Serum concentrations of ritodrine or β-receptor activity might be of value in future investigations.

Potassium levels drawn before therapy and every 6 hours thereafter showed a resolution toward pretreatment levels without potassium supplementation. There was no relationship between serum potassium concentrations and electrocardiographic changes (Table I).

Comment

β-Adrenergic stimulators are not as specific in vivo as they are in vitro, and therefore both β_2 and β_1 receptors are stimulated by the tocolytics that are presently in clinical use. This incomplete in vivo affinity results in multiple major and minor adverse side effects.

Myocardial ischemia in symptomatic patients has been reported by several authors[1, 3, 4, 6] and electrocardiographic changes in one asymptomatic patient were reported by Michalak et al.[7] Based on these reports of maternal cardiovascular morbidity and even mortality, many practitioners have chosen to evaluate all symptomatic patients with electrocardiograms. The results of these evaluations have been used as criteria to continue or discontinue tocolytic therapy.[8] Specifically, ST segment depression and T wave flattening or inversion could be considered indicative of myocardial dysfunction necessitating cessation of treatment. However, the frequency of electrocardiographic changes in asymptomatic patients undergoing tocolytic therapy has not been previously established for ritodrine. With use of a different β-adrenergic agent, German investigators[9] noted similar findings to those described by us in this study. Wiest et al.[9] showed that ST segment depression and T wave flattening or inversion were present in a majority of women treated with fenoterol for preterm labor. Furthermore, the same authors reported that serum concentrations of serum glutamic oxaloacetic transaminase and lactate dehydrogenase in those women who had electrocardiographic changes were normal and thus probably not representing a significant myocardial damage.[9] In other reports, measurements of the MB isoenzyme fraction of creatinine phosphokinase in patients with electrocardiographic changes have shown no elevations.[10, 11]

In this study we have shown that a majority of women undergoing ritodrine therapy did develop electrocardiographic changes that suggested myocardial ischemia, but they remained asymptomatic. Therefore no obvious relationship between symptoms of myocardial ischemia and electrocardiographic changes could be established. The physiologic significance of these changes could be explained by a maternal adaptation to the

ritodrine-induced tachycardia. Relative hypoperfusion of the subendocardium may cause subtle changes in the maternal electrocardiograms even in the absence of functional ischemia and enzyme abnormalities. The normal 1:1 perfusion ratio of epicardium to subendocardium may be altered by heart rate changes. Development of a sinus tachycardia is often accompanied by a relative decrease in blood flow to the subendocardium.[6] This is considered a physiologic adaptation, which has also been noted to occur during exercise-induced tachycardia and has no known pathologic significance. However, this rate-induced change in myocardial perfusion ratio has never been associated with changes in more sensitive indicators of myocardial damage such as serum cardiac enzyme elevations.[9]

Ritodrine-induced hypokalemia evolves from movement of potassium into the intracellular space.[8, 12, 13] Numerous investigators have shown that the serum potassium level drops sharply in the first 6 to 8 hours of therapy without an increase in urinary potassium excretion.[8, 12, 13] However, potassium concentration returns toward normal without supplementation by 24 hours of therapy.[8, 12, 13] Since the electrocardiographic changes seen in this study were not associated directly with degree of hypokalemia, a relationship between serum potassium concentration and the presence of electrocardiographic changes could not be established.

In conclusion, our study has shown that electrocardiographic changes in a sample of patients undergoing ritodrine therapy may be a frequent event. No relationship between symptoms of ischemia and electrocardiographic changes could be seen. We suggest that these changes could be physiologic expressions of ritodrine-induced maternal tachycardia and are not likely to be representative of myocardial ischemia. Based on our present data, we doubt the validity of the use of electrocardiograms of asymptomatic patients as a sole criterion for discontinuation of β-adrenergic therapy. Additional cardiovascular evaluations such as echocardiograms and serum cardiac enzyme concentration tests are necessary to establish the true significance of the electrocardiographic changes in the asymptomatic patient.

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Ritodrine-induced neutropenia

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Ritodrine hydrochloride, a β-sympathomimetic treatment for premature labor, has been associated with the development of pulmonary edema, various metabolic derangements, myocardial ischemia, and infarction. We present the first reported case of absolute neutropenia after prolonged intravenous administration of ritodrine with rapid, spontaneous reversal once the medication was discontinued. (Am J Obstet Gynecol 1986;154:924-5.)

Key words: Ritodrine hydrochloride, neutropenia, tocolysis

β-Adrenergic receptor agonists have been widely used for tocolysis for many years. Although ritodrine can be used with relative safety while pulse rate, respirations, blood pressure, serum glucose, potassium, and hematocrit are monitored, serious side effects have been reported. The drug has been associated with the development of pulmonary edema, myocardial ischemia, ketoacidosis, hypocalcemia, and hyperlactacidemia.[1] We did not find any reports of hematologic derangements with ritodrine in the literature.

Case report

A 22-year-old Hispanic woman, gravida 3, para 1, abortus 1, was admitted elsewhere for premature labor at 33 weeks' gestation. She was begun on a regimen of a continuous intravenous infusion of ritodrine, and contractions ceased. Other than some nausea and palpitations, she tolerated the medication without difficulty. Numerous attempts were made to wean the patient off intravenous ritodrine and maintain her on a regimen of oral medication, but contractions resumed each time. The intravenous infusion varied from 0.15 to 0.3 mg/min.

On admission the white blood cell count was 8900/mm³, with 77% segmented neutrophils, 22% lymphocytes, and 1% monocytes. Hemoglobin was 13.3 gm/dl. Three weeks later the white blood cell count had fallen to 3700/mm³, with 72% neutrophils and 37% lymphocytes. The white blood cell count eventually dropped to 2100/mm³, with 2% segmented neutrophils, 2% band forms, 63% lymphocytes, 2% monocytes, and 1% basophils. Hemoglobin remained 11 to 12 gm/dl, and platelet count also stayed in the normal range. When a nadir of absolute neutropenia with neutrophils of 84/mm³ was reached, she had been receiving intravenous ritodrine for 26 days and had received no other medication. Ritodrine was discontinued and she was transferred to our university hospital. Because she was in labor, a repeat cesarean section was performed without complications at 37 weeks of gestation. On physical examination no splenomegaly was noted, and no sites of infection were identified.

The patient had an uneventful postoperative course and no postpartum infectious complications occurred. Three days after operation, the white blood cell count had risen to 3700/mm³, with 39% neutrophils, 24% bands, 30% lymphocytes, 15% monocytes, and 1% basophils. At a checkup 6 weeks postpartum all hematologic indices remained in the normal range. The baby was normal, left the hospital with the mother on the fifth postpartum day, and has had no developmental difficulties.

Comment

This is the first report of neutropenia developing in a patient after almost 1 month of intravenous ritodrine for tocolysis, given in the recommended doses. The patient had no history of leukopenia or recent exposure to another drug or toxin. The leukopenia and neutropenia resolved very rapidly, within 3 or 4 days, after cessation of ritodrine. Bone marrow examination was not performed because of this rapid resolution. Rechallenge with ritodrine also was not carried out because of the risk of neutropenia and the possible necessity for prolonged intravenous administration. Follow-up 3 years later has not revealed any recurrent neutropenia or blood disorder.

Drug-induced agranulocytosis may be the result of decreased precursor cell proliferation, cell loss in the maturation process, increased margination in the circulation, increased peripheral destruction, or a combination of these mechanisms.[2] The pathophysiologic mechanisms of drug actions leading to neutropenia are poorly understood. Both toxic effects of drugs on neutrophil formation and immunologic mechanisms causing accelerated cell destruction have been proposed and demonstrated in a few instances.

In this instance the neutropenia was probably a non–

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dose-related idiosyncratic reaction. The slowly declining neutrophil count and rapid recovery after drug withdrawal in this case suggest suppressed neutrophil production or production of antibodies leading to immunologic injury of neutrophils. The exact mechanisms by which ritodrine may induce neutropenia are not known but this complication needs to be considered in cases where long-term administration of ritodrine is contemplated.

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Meningoencephalitis caused by *Histoplasma capsulatum* complicating pregnancy

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We report what appears to be the first established case of disseminated histoplasmosis in pregnancy. Involvement of the central nervous system made treatment difficult, and the course of disease required iatrogenic preterm delivery of an uninfected fetus. Aspects of pathogenesis, diagnosis, interaction with pregnancy, and treatment for this common infection, hitherto unreported in pregnancy, are presented. (Am J Obstet Gynecol 1986;154:925-31.)

Key words: Pregnancy, meningoencephalitis, meningitis, *Histoplasma capsulatum*, histoplasmosis

Histoplasmosis is a frequently asymptomatic mycosis caused by the yeast phase of the dimorphic fungus *Histoplasma capsulatum*.[1] The disease is endemic within the Mississippi, Missouri, and Ohio river basins in the United States. Histoplasmosis occurs outside North America and was initially described by Darling in 1906 among natives of the Panama Canal Zone. Although immunologic studies suggest that up to 50% of adults living within endemic areas have had histoplasmosis, there are only isolated reports of healed, asymptomatic, or chronic pulmonary histoplasmosis that were identified antenatally or at time of autopsy for choriocarcinoma.[2-4] There do not appear to be other reports of disseminated histoplasmosis occurring during pregnancy.[1, 5] We report the first such observation in a 26-year-old, primigravid Panamanian woman, whose pregnancy was complicated by fatal *H. capsulatum* meningoencephalitis necessitating delivery of an 810 gm preterm infant by cesarean section.

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Case report

A 26-year-old Panamanian woman, gravida 1, para 0, was admitted at 26 weeks of gestation to the psychiatric ward of her local hospital with a diagnosis of "psychosis due to pregnancy" because of a 3-month history of headaches and 3 weeks of increasing confusion. She had immigrated to the United States from her native Panama as a United States Army dependent 6 months before her illness and had not traveled after her arrival in Colorado. Her father had expired of miliary tuberculosis 2 years before her arrival in this country. The patient's previous tuberculin skin test status was unknown. Previously she had been healthy and appeared well nourished. There was no known exposure to blood products, parenteral substance abuse, or exposure to individuals at risk for acquired immune deficiency syndrome. During this 5-day hospitalization, the patient developed right seventh nerve palsy and increasing lethargy ending in coma. A tuberculin skin test was positive (2 cm induration), but histoplasmin and coccidiodin tests were negative. Cerebral spinal fluid examination showed a red blood cell count of 3850/mm^3, white blood cell count of 110/mm^3 (segmented neutrophils 30%, lymphocytes 59%, and monocytes 11%) with protein, 546 mg/dl, and glucose, 10 mg/dl. Peripheral white blood cell count was 15,000/

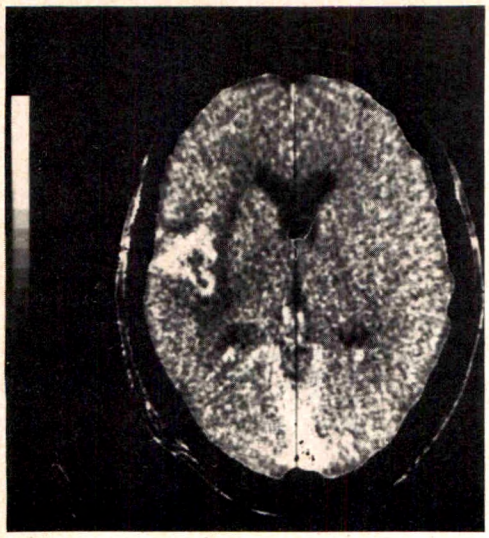


Fig. 1. Computed tomographic scan demonstrating recent infarction of right perisylvian area. See Fig. 2 for correlation.

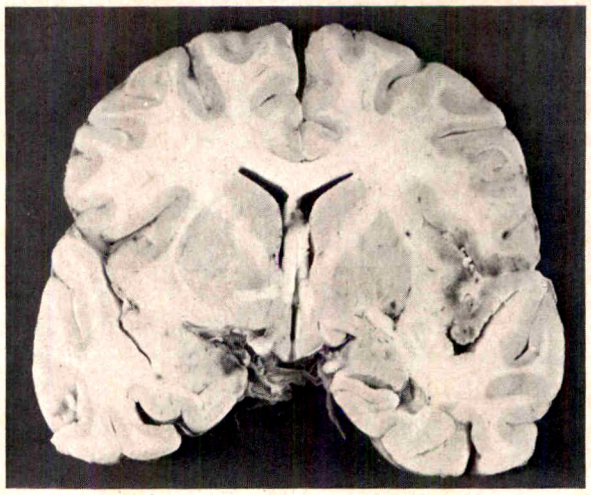


Fig. 2. Coronal brain section at the level of the anterior commissure illustrating the discolored infarctions of the right perisylvian cortex and the left amygdala. The cracking and separation between devitalized and intact tissue in the right superior temporal gyrus are artifactual and common in infarctions occurring several days earlier. (×80.)

mm³, with segmented neutrophils 86%, bands 1%, lymphocytes 9%, and monocytes 4% with an erythrocyte sedimentation rate of 89 mm/min. Chest roentgenogram and other laboratory tests were within normal limits. The patient was transferred to the University of Colorado Health Sciences Center and treated for presumed tuberculous meningitis in pregnancy. She was treated with parenteral isoniazid, ethambutol, rifampin, and supplementary pyridoxine and multisystem support. Computed tomograms demonstrated acute grade II/V hydrocephalus with probable infarction of the sylvian region compatible with obstruction of the right middle cerebral artery (Fig. 1). These findings were confirmed by a cerebral angiogram. A decompressing ventriculoperitoneal shunt was placed, with no clinical improvement. Subsequent computed tomograms demonstrated changes compatible with further infarction of anterior lobe tissues supplied bilaterally by the anterior cerebral arteries. Focal right-sided and grand mal seizures developed and were treated with phenobarbital and Dilantin. Serial cerebrospinal fluid examinations of stained smears, india ink preparations, and bacterial, mycobacterial, fungal, and viral cultures had been negative.

On the twelfth day of hospitalization cerebrospinal fluid IgM-histoplasma antibody obtained at the referring hospital was reported as positive. Antituberculous medications were discontinued, and administration of intravenous amphotericin B was begun. The following day, previously obtained cerebrospinal fluid fungal cultures yielded multiple colonies of mold compatible with *H. capsulatum*, confirming the diagnosis of histoplasma meningitis. The mother subsequently suffered a stormy course marked by aspiration pneumonitis requiring intubation and intensive respiratory support. A bronchopulmonary fistula with pneumothorax and pneumomediastinum developed, requiring placement of two chest tubes.

The pregnancy had appeared to be uncomplicated before the onset of symptoms. On admission, examination of clinical dating parameters, uterine size, and fetal ultrasound measurements were compatible with 26 weeks' gestation. The fetus was vertex, and the cervix was long and closed. The fetus was monitored with periodic ascultation and three-times-weekly nonstress testing with an external monitor. Coincident with maternal deterioration, uterine contractions were noted and treated with magnesium sulfate, 2 gm/hr intravenously. Two doses of 12.5 mg of betamethazone were given intramuscularly. Ninety-six hours later, on the fourteenth hospital day, concurrent with further maternal respiratory deterioration and worsening hypoxemia, uterine contractions resumed, and fetal monitoring showed sustained fetal tachycardia of 160 to 170 bpm and repetitive late decelerations. A biophysical profile with ultrasound determined the presence of amniotic fluid, decreased tone, and no fetal movement or breathing. Measures were taken to improve maternal oxygenation, but no change was observed in fetal findings; a primary cesarean section was performed. A 810 gm male infant of 27 to 28 weeks' gestation was delivered (Apgar scores of 3 at 1 minute and 3 at 5 minutes) and immediately intubated in the delivery room. The infant developed hyaline membrane disease and required ligation of a patent ductus arteriosus. At 9 months of age, the infant has chronic lung disease necessitating respiratory support. Shortly after birth and before routine administration of vitamin K, the infant developed bleeding from catheter sites. Laboratory workup and treatment results were consistent with vitamin K deficiency; vitamin K was given. The placenta was grossly and histologically normal. Bacteriologic and fungal cultures of the placenta were negative. Cord blood and urine obtained at 2 weeks of age

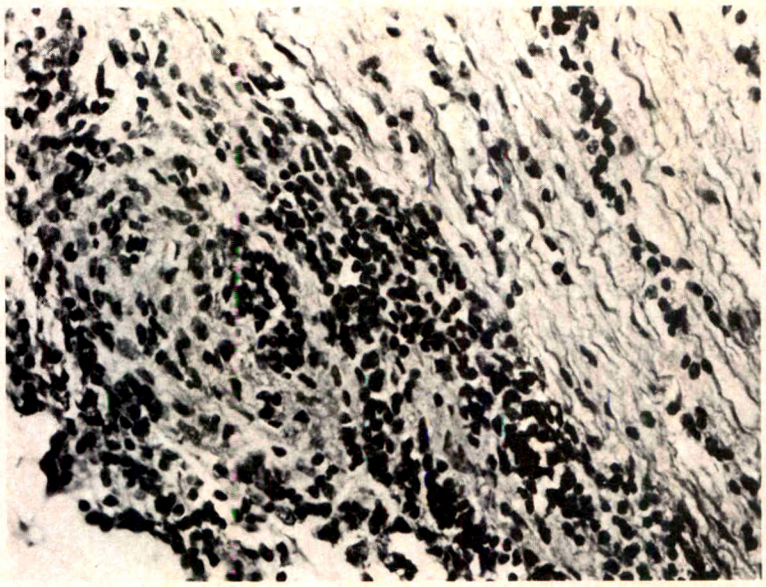


Fig. 3. Histiocytic and lymphocytic inflammation with ill-defined granuloma formation in leptomeninges and parenchyma of the left third cranial nerve. (Hematoxylin and eosin; ×325.)

were negative for IgM-antihistoplasma antibody and antigen.

After delivery the mother transiently improved but died without gaining consciousness on the twenty-fourth hospital day from respiratory insufficiency secondary to massive bronchopulmonary air leaks and aspiration pneumonitis.

Autopsy findings

Necropsy was limited by permit to biopsy sampling of organs. The unfixed brain weighed 1260 gm and demonstrated mild opacification of the meninges with gyral flattening most prominent over the base. Freshly cut coronal sections revealed a recent softened, noncavitated cortical infarction in the right perisylvian region (Fig. 2). A smaller similar cortical infarct was noted in the left amygdala (Fig. 2). The lateral, third, and fourth ventricles were mildly compressed by edema. Microscopically the meninges showed widespread plasmacytic, histiocytic, and lymphocytic inflammation with secondary involvement of cranial nerves, as well as the media and adventitia of adjacent vessels (Fig. 3). Vessels overlying the right perisylvian infarct were encompassed by caseous necrosis (Fig. 4), and some showed mild reactive intimal proliferation (Fig. 5). The infarct showed abundant macrophage infiltration and proliferated capillaries and appeared to be several weeks in age. Microscopic infarctions were also noted in the hippocampus, parahippocampal gyrus, cerebellum, midbrain tectum, and pontine tegmentum. Staining with Grocott methenamine silver demonstrated numerous small budding yeast in the leptomeninges, vessels, cranial nerves, and infarcted brain tissue (Fig. 6). Bacterial, viral, fungal, and mycobacterial cultures were negative except for *H. capsulatum*. Examination of the lungs demonstrated diffuse congestion and consolidation and a bronchopulmonary fistula in the right upper lobe. A single enlarged caseous node in the left lung hilum was found to be centrally necrotic with peripheral fibrosis but without giant cells or calcification. Methenamine silver–stained sections of this node demonstrated abundant small budding yeast forms of histoplasmosis (Fig. 6). Examination of lung parenchyma revealed numerous foamy macrophages in alveolar spaces along with hyperplastic alveolar lining cells and interstitial fibrosis, but no yeast forms were noted. Except for the healing postpartum uterus and a congested liver, all other organs were grossly and microscopically unremarkable; examination included negative special stains for fungi and mycobacteria.

Comment

The hypheal form of *H. capsulatum* exists in soil—as well as in bird, bat, and other small mammalian excreta—in endemic areas. Histoplasmosis is acquired by the inhalation of macroconidia or microconidia or mycelial fragments of *H. capsulatum* in dust. The lower respiratory tract and the reticuloendothelial system are the primary sites of infection. *H. capsulatum* may disseminate within lymph or blood, or perhaps yeast-laden macrophages, to involve liver, spleen, central nervous system, and other body sites.

Despite the frequency of occurrence of histoplasmosis within endemic areas and the presumed infection among pregnant women living in these areas, little is known regarding interactions between this potentially severe fungal infection and pregnancy. Wheat's un-



Fig. 4. Necrotic leptomeningeal vessel overlying the right perisylvian infarction and encompassed by caseation necrosis. (Hematoxylin and eosin; ×130.)

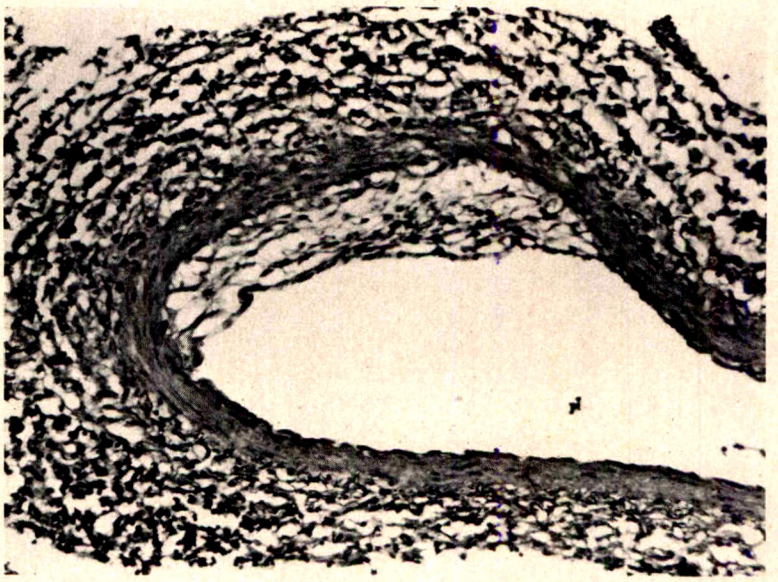


Fig. 5. Small leptomeningeal vessel from the region of the infarction demonstrating intense mononuclear cell inflammation in the outer vessel wall and early reactive intimal proliferation. (Hematoxylin and eosin; ×130.)

published observations of 175 Indiana women with histoplasmosis included seven pregnant individuals who may be added to previously reported women with histoplasmosis and intercurrent pregnancy.[5] These initial observations and the paucity of previous medical reporting of histoplasmosis in pregnancy suggest that dissemination during the immunobiologic alterations of pregnancy is not a feature of histoplasmosis.

In contrast, dissemination of coccidioidomycosis in women occurs almost entirely during pregnancy, and the later coccidioidomycosis is acquired during pregnancy, the greater the frequency of dissemination.[6, 7] Dissemination of coccidioidomycosis during pregnancy is also more likely to be fatal.[7] *Coccidioides immitis* is the only fungi reported to cross the placenta and cause hematogenous infection of the fetus.[7] At least 10 reports of cryptococcosis and four cases of North American blastomycosis in pregnant women have been reported.[8, 9] One of four infants born to women with active blastomycosis acquired this infection.[10] Although it was suggested that the infant acquired the fungal infection through transplacental transmission, the

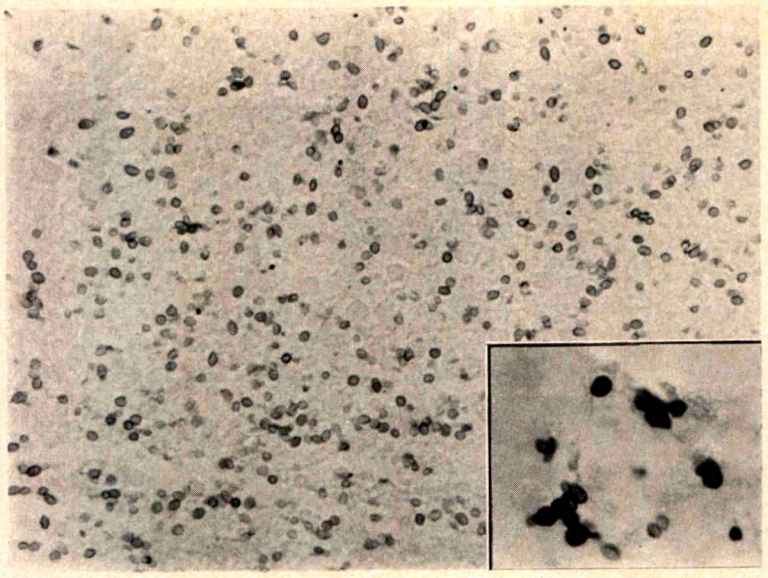


Fig. 6. Numerous *H. capsulatum* found in the caseous hilar lymph node. Inset: The budding yeast seen at higher power was also easily demonstrable in the leptomeninges. (Grocott methenamine silver; ×325. Inset: ×850.)

mother had *Blastomyces dermatitides* in the urine, and the perinate may have aspirated infectious material at birth. Both histoplasmosis and blastomycosis genital infections from sexual contact have been reported.[11, 12] Lack of placental and/or fetal infection in this case of disseminated histoplasmosis confirms the observation that visceral fungal infections, including histoplasmosis in the absence of birth canal infection, do not put the fetus at high risk for intrauterine infection. Unexplained anatomic or immunologic attributes of trophoblastic tissue may account for the observed rarity of placental fungal infection.

Cell-mediated immunity. An integrated immune response relying primarily on cell-mediated immunity protects the body from invasion by fungi.[13] Limited observation of nonpregnant patients with healed histoplasmosis does not demonstrate increased risk of exacerbation or dissemination during immunosuppression. On the other hand, treatment with corticosteroids or other immunosuppressive agents increases the risk of dissemination and death in active histoplasmosis and coccidioidomycosis and also increases the risk of acquiring blastomycosis. Pregnancy induces a state of relative immunosuppression that includes decreased (1) numbers of helper T cells, (2) natural killer cell activity, (3) lymphocyte blastogenic responses, (4) chemotaxis, (5) rejection of skin allografts, and (6) delayed-type skin test reactivity.[5] These immune alterations are most prominent in the third trimester of pregnancy and may account for the observed worsened course of blastomycosis late in pregnancy.[5, 6]

Invasive fungal infections, including histoplasmosis, have been documented to independently mediate systemic immunosuppression.[14-15] Fungal infection–induced immunosuppression is nonspecific and requires the ongoing presence of organisms. Apparently the immunosuppression is mediated by suppressor cells.[13-15]

Our patient had not been in an endemic area for 6 months and presumably had acquired histoplasmosis in her native Panama, since histoplasmosis is very rare among natives of nonendemic areas such as Colorado. Despite the negative admission chest roentgenogram, the histologic finding of fungal forms consistent with *H. capsulatum* in this patient's lung granuloma suggests that the histoplasmosis infection, although contained, was ongoing and originally had a primary pulmonary focus. The histoplasmosis infection may have mediated a degree of immunosuppression that was additive with the effect of pregnancy, as well as serving as a source of dissemination to the central nervous system. Although it is difficult to be sure, both the rarity of histoplasmosis in Colorado and the patient's prolonged history of headaches suggest that the woman acquired the infection in Panama and that symptoms only became apparent when the infection spread to the central nervous system during the physiologic immunosuppression of pregnancy.

Diagnosis. Diagnosis of the various expressions of *H. capsulatum* infection is frequently difficult. Awareness of the varied expression of histoplasmosis remains the prerequisite for the diagnosis of this and other fungal infections.[1] A history of exposure to environmental sources is infrequent, and physical and roentgenographic findings are not specific.[1] Histoplasmin antigen skin testing or complement-fixation serologic tests have not proved useful, especially in disseminated histo-

plasmosis.[16] Microbiologic and/or pathologic identification are essential for reliable diagnosis.[1] With repeated cultures of sputum and cerebrospinal fluid, nearly all instances of active lung or meningeal infection will yield *H. capsulatum*.[1, 17] Any biopsy specimens taken from a patient in whom histoplasmosis is a possibility should be cultured for fungi and microscopically examined after staining with methenamine silver. Examination of cerebrospinal fluid commonly demonstrates findings similar to tuberculosis meningitis with increased levels of protein, hypoglycorrhachia, and a predominance of lymphocytes and monocytes.[17] Recently developed assays for *H. capsulatum* antigen-specific IgM or IgG antibody in body fluids (blood, urine, cerebrospinal fluid, etc.) are highly promising but remain to be fully evaluated. In this patient, cerebrospinal fluid findings and a positive tuberculin skin test suggested tuberculosis, but cerebrospinal fluid fungal culture and cerebrospinal fluid anti–histoplasmosis IgM confirmed the diagnosis.

Treatment. Treatment of any central nervous system fungal infection is difficult, since no antifungal agent crosses blood/brain barriers adequately. Optimal therapy for disseminated histoplasmosis requires completion of a full course of intravenous amphotericin B (1.5 to 2.5 gm).[17] The role of alternative or concomitant treatment with other antifungal agents, including ketoconazole, 5-fluorouracil, and miconizole, remains unsettled. None of these agents are likely to surpass the treatment efficacy of amphotericin B.[1] The analysis of Ismail and Lerner of over 20 mother-fetus pairs demonstrates that amphotericin B crosses the placenta and reaches roughly one third of maternal levels within the fetus without evidence of toxicity or teratogenesis.[18] These authors noted that adverse effects of amphotericin B, including nephrotoxicity with azotemia and/or hypokalemia, appear no worse in pregnant than in nonpregnant patients.[18] Ketoconazole has the practical advantages of oral administration but may be inadvisable during pregnancy on the basis of observed teratogenecity in laboratory animals and transient inhibition of host–steroid hormone synthesis.[19] Rifampin, used here as therapy for presumed *Mycobacterium tuberculosis* infection, enters the central nervous system but is of unproven benefit for fungal central nervous system infection. Rifampin appears safe in pregnancy after organogenesis is completed. As noted in this case, maternal rifampin (and phenytoin) administration may be associated with neonatal coagulopathy, which is reversible with maternal or neonatal vitamin K administration.[20, 21] Experience with any therapy of invasive fungal infection among pregnant women and their progeny remains limited. Surveillance for fetal well-being with contemporary methods of fetal evaluation should be performed during treatment. Authorities agree that treatment of histoplasmosis with amphotericin B is indicated only in patients who have the potential for prolonged illness or death.[1]

In summary, we have described the occurrence of disseminated histoplasmosis with spread to the central nervous system in a pregnant woman who had immigrated from an endemic area 6 months before the recognition of illness. Pregnant women are increasingly frequent travellers and are thus exposed to varied endemic illnesses worldwide. Careful history-taking for travel or residence in different geographic regions is frequently crucial for the timely diagnosis and treatment of many infectious diseases, including histoplasmosis. Incompletely understood immunologic alterations induced by pregnancy and ongoing histoplasmosis probably played roles in this woman's progressive illness. Cesarean section of a premature infant was mandated because of fetal distress caused by ultimately fatal maternal respiratory failure. Delay in accurate diagnosis of histoplasma-mediated meningoencephalitis impaired treatment. Pregnancy-associated immune alterations are documented to potentiate fungal infections such as coccidioidomycosis and blastomycosis. This case suggests that active histoplasmosis may also be aggravated, on occasion, during pregnancy.

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Sequential nonstress testing with use of each fetus as its own control

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The sequential nonstress tests of 126 high-risk fetuses were assessed for baseline fetal heart rate, acceleration frequency, amplitude, and duration and the observance of fetal heart rate decelerations. In 108 fetuses (563 tests) with normal perinatal outcomes, no significant trends in these parameters were found. In 18 fetuses (104 tests) with perinatal compromise, 16 had significantly declining acceleration frequencies, 15 had decreasing acceleration duration, four had rising mean baseline rates, and seven, in their last test before delivery, had repetitive late or severe variable decelerations. The last tests of 11 of these 18 fetuses met institutional criteria for reactivity. When the nonstress tests of compromised fetuses were viewed sequentially, acceleration frequency declined by an average of 59% and acceleration duration by an average of 40%. Sequential assessment of the nonstress test, when compared with the use of standard reactivity criteria, improved test sensitivity from 39% to 89% and negative predictive value from 91% to 98%. We conclude that sequential nonstress test assessment in individual fetuses may improve the diagnostic value of this modality and lead to earlier recognition of fetal compromise. (AM J OBSTET GYNECOL 1986;154:931-6.)

Key words: Nonstress test, high-risk fetuses, sequential testing, fetal compromise

Fetal heart rate (FHR) patterns in the third trimester are characterized by stable long-term baseline rates, cyclic oscillations in baseline rate, and movement-associated FHR accelerations. Sequential FHR changes, for example, decreased frequency of movement-associated FHR accelerations, loss of baseline variability, and the appearance of late FHR decelerations, may precede overt fetal compromise. However, there are limited human data on the magnitude of these changes needed to achieve diagnostic significance or the order in which they evolve in antepartum FHR tracings. Experimental evidence, obtained from chronic animal preparations,[1-4] suggests that hypoxic stress may have a variable impact on the FHR baseline, ranging from mild tachycardia, the loss of reactive accelerations, and altered FHR variability to the evolution of late decelerations and bradycardia. These data, from different species and under varied experimental conditions, are difficult to compare and apply directly to clinical antepartum testing.

The nonstress test is a means of providing rapid assessment of fetal condition.[5-7] Clinical interpretation is based on the presence or absence of movement-associated FHR accelerations, and individual tests are used in diagnosis. Typically this approach does not consider the relationship of previous nonstress tests to subsequent tests and has been criticized for its failure to detect the early appearance of fetal compromise.[8] Sequential comparison of nonstress tests, with use of each

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Table I. Test indications for the study population

Diagnosis	*n*	%
Postdatism	24	19.0
Suspected intrauterine growth retardation	24	19.0
Hypertension	30	23.8
Preeclampsia	16	12.7
Diabetes mellitus (Class A-D)	20	15.9
Rh sensitization	2	1.6
Miscellaneous medical complications	10	7.9

fetus as its own control, is an alternative approach that has not been adequately explored.

We undertook this study to examine selected components of the nonstress test to determine whether there were significant sequential trends in any of the nonstress test parameters (mean baseline rate, the frequency, amplitude, or duration of movement-associated FHR accelerations, or the presence of decelerations) which could be used to improve the ability of the nonstress test to provide evidence of fetal jeopardy.

Material and methods

One hundred twenty-six fetuses were examined in the antenatal assessment unit of the Medical College of Georgia Hospital and Clinics. All fetuses were at least 33 weeks' gestational age, had no ultrasound evidence of major anomalies, and were tested for the reasons listed in Table I.

Testing was performed in a standardized manner with use of a previously reported institutional protocol.[7] All tests were conducted for a minimum of 30 minutes, with use of Hewlett-Packard 8040A electronic monitors in the Doppler mode. Fetal movements were entered by means of a remote event indicator and were corroborated by experienced observers. The nonstress tests were analyzed retrospectively with the investigators blinded to perinatal outcome. The following parameters were assessed: (1) mean 1-minute baseline heart rate, exclusive of accelerations, decelerations, or signal loss; (2) frequency of movement-associated FHR accelerations exceeding 15 bpm amplitude and 15 seconds' duration; (3) mean movement-associated FHR acceleration amplitude and duration; (4) the presence of late or variable decelerations.

The following perinatal outcome parameters were considered: (1) mortality, (2) intrapartum fetal distress, (3) 5-minute Apgar score <7, (4) neonatal acidemia (umbilical arterial pH <7.20), and (5) intrauterine growth retardation.

Statistical analysis included χ^2 tests, *t* tests, runs tests, and signed rank test where appropriate; a p value of <0.05 was considered significant.

Results

Six hundred sixty-seven nonstress tests were performed on the 126 fetuses, at intervals of ≤7 days. All studies contained <10% loss of signal Each fetus underwent at least five serial nonstress tests (mean: 5.3) and was delivered within 1 week of the last test.

One hundred eight fetuses, producing 563 nonstress tests, had normal perinatal outcomes, that is, no perinatal deaths or intrapartum or neonatal complications. Eighteen fetuses had one or more of the perinatal complications listed above and underwent 104 nonstress tests. The obstetric characteristics of these two groups are compared in Table II. There were significant differences in gestational age at delivery and mean birth weight between the groups.

The runs test was applied to the individual FHR parameters of the sequential tracings from both groups. In the normal group, no statistically significant trends were noted in any nonstress test parameter (mean baseline FHR, movement-associated FHR acceleration frequency per 30 minutes, movement-associated FHR acceleration amplitude and duration). No late decelerations occurrred with spontaneous uterine contractions; 8 fetuses exhibited mild, transient variable decelerations.

Table III summarizes the pregnancies with abnormal outcomes. Sixteen of 18 fetuses had significantly declining frequencies of movement-associated FHR accelerations (Fig. 1, runs tests, $p < 0.01$). The mean decline in movement-associated FHR acceleration frequency was 59%. Although no significant trends in movement-associated FHR acceleration amplitude were noted, 15 fetuses in the abnormal group had significant declines in the mean duration of movement-associated FHR accelerations (Fig. 2, runs tests, $p < 0.01$). The mean decline in movement-associated FHR acceleration duration was 39.9%. Four of the abnormal fetuses had significantly rising FHR baselines during the sequence of their nonstress tests (Fig. 3, runs tests, $p < 0.01$). Seven of these 18 fetuses (39%) also had late or deep variable decelerations on their last tracing before delivery. Sixty-one percent (11 of 18) of these last tracings would have been classified as "reactive" by our institutional criteria, that is, three accelerations (>15 bpm, >15 seconds) in 30 minutes.[7]

Nonstress test sensitivity with use of conventional criteria was 39% but increased to 89% when the nonstress test parameters were assessed sequentially. Nonstress test specificity was 100% by both approaches. These differences were significant (signed rank testing, $p < 0.01$). Nonstress test positive predictive values were 100% by either approach, and negative predictive values were 91% (conventional criteria) and 98.2% (sequential parameters). These latter figures closely ap-

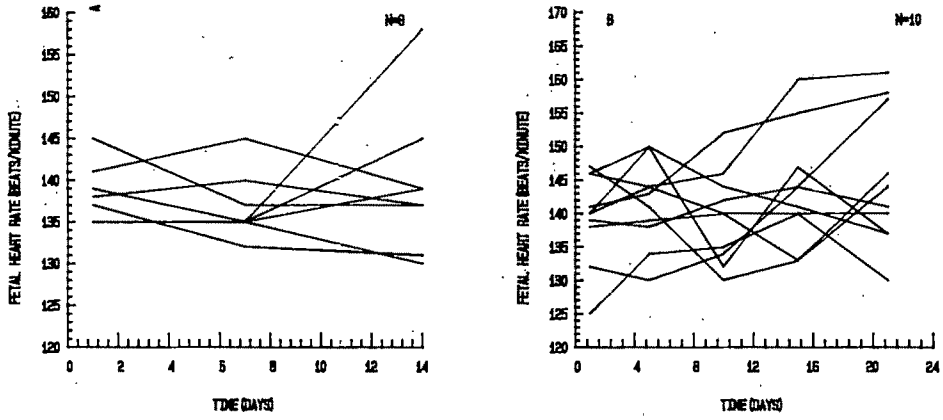


Fig. 1. *Panels A* and *B* show the trends in the acceleration frequencies in sequential nonstress tests of fetuses with abnormal outcomes, which were studied over 2-week and 3-week intervals, respectively. Sixteen of the 18 fetuses had significant declines in acceleration frequency (runs tests, $p < 0.01$).

Table II. Obstetric characteristics of the study group

	Normal outcome	*Abnormal outcome*	*p**
No. of patients	108	18	—
Mean no. of tests per patient (±SD)	5.2 ± 1.4	6.2 ± 2.6	NS
Mean birth weight (gm) (±SD)	3328 ± 597	2324 ± 703	<0.0001
Mean gestational age at delivery (wk) (±SD)	39.4 ± 2.1	37.7 ± 3.1	<0.01

*Student's *t* test.

proached but did not reach significant differences (signed rank tests, $p = 0.06$).

Comment

Fetal compromise may result from a variety of intrauterine insults. Antepartum FHR testing has been proposed as a means of detecting the presence of fetal hypoxia and possible asphyxia. Natale et al.,[2] with use of a chronic sheep model, have shown that acute hypoxia results in a markedly reduced incidence of fetal body movements. As FHR accelerations are largely triggered by fetal movements in normal third-trimester pregnancies,[10] a significant fall in the number of movement-associated FHR accelerations during subsequent nonstress tests could be a useful premonitory sign. Declines in movement-associated FHR acceleration frequency were the most consistent finding in our series and actually preceded all instances where late or severe variable decelerations subsequently occurred. In most abnormal fetuses (14 of 16) significant decreases in movement-associated FHR acceleration frequencies were accompanied by declines in movement-associated FHR acceleration duration but not amplitude. Although Ingardia et al.[9] reported that fetuses with subsequently positive contraction stress tests had quantitative differences in movement-associated FHR acceleration amplitude and duration when compared with those producing negative contraction stress tests, they did not find significant differences in the number of accelerations or fetal movements between these two groups. However, their observations were not based on sequential trends in the nonstress test. In our series, 61% of cases with abnormal outcomes met the minimum criteria for reactivity, despite a mean decline in movement-associated FHR acceleration frequency of 59%. In all instances where movement-associated FHR accelerations were present on the final tracing, their mean duration exceeded 15 bpm, although there was a mean decline in movement-associated FHR acceleration duration of 40%.

Mild acute fetal hypoxemia may also produce fetal tachycardia, as shown by Towell et al.[1] Although a mean FHR baseline exceeding 155 bpm evolved in the sequential tracings of four compromised fetuses, it was always accompanied by significant declines in movement-associated FHR acceleration frequency and duration. The significance of sequential elevation of the FHR baseline would require confirmation by larger numbers of fetuses studied with umbilical arterial blood gases.

The principle of the use of individual fetuses as their own controls has been applied to the daily fetal movement counts[11] and maternal serum estriol measurements.[12] Our data suggest that normal third-trimester

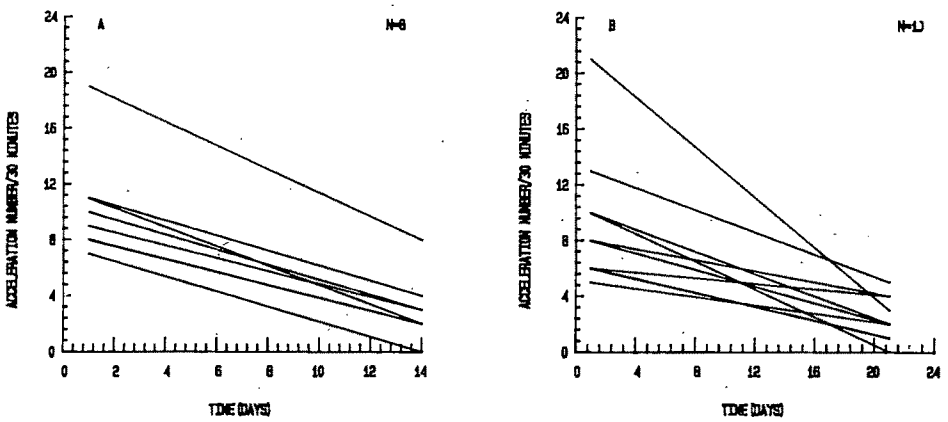


Fig. 2. *Panels A* and *B* show the trends in mean acceleration duration in sequential nonstress tests of fetuses with abnormal outcomes, which were studied over 2-week and 3-week intervals, respectively. Fifteen of the 18 fetuses had significant declines in acceleration duration (runs tests, $p < 0.01$).

Table III. Summary of fetuses with abnormal outcomes

Patient No.	*Clinical problem*	*Interval from last test to delivery (hr)*	*No. of tests*	*Mean baseline rate**	*Acceleration frequency*	*Mean acceleration amplitude*	*Mean acceleration duration*
1	Intrauterine growth retardation	<24	9	NS	↓	NS	↓
2	Postdatism	<24	5	NS	NS	NS	↓
3	Postdatism	<24	5	NS	↓	NS	↓
4	Hypertension	<24	5	↑	↓	NS	↓
5	Hypertension	<24	5	NS	↓	NS	NS
6	Intrauterine growth retardation	<24	5	↑	↓	NS	↓
7	Diabetes mellitus	<24	5	↑	↓	NS	↓
8	Postdatism	<24	5	NS	↓	NS	↓
9	Intrauterine growth retardation	<24	5	NS	↓	NS	↓
10	Preeclampsia	<24	6		↓	NS	NS
11	Intrauterine growth retardation	48	6	NS	↓	NS	↓
12	Hypertension	48	5	NS	↓	NS	↓
13	Hypertension	72	5	NS	NS	NS	↓
14	Hypertension	24	5	NS	↓	↑	↓
15	Hypertension	<24	5	NS	↓	NS	NS
16	Postdatism	<24	5	↑	↓	NS	↓
17	Hypertension	<24	12	NS	↓	NS	↓
18	Rh sensitization	<24	5	NS	↓	NS	↓

*NS: No significant change; ↑ (↓): significant increase (decrease), runs test, $p < 0.05$.
‡CS: Cesarean section, VD: vaginal delivery.

fetuses, studied over intervals of 2 to 3 weeks, do not exhibit significant changes in the frequency, duration, or amplitude of movement-associated FHR accelerations. Under similarly controlled testing conditions, compromised fetuses often exhibit progressive declines in movement-associated FHR acceleration frequency and duration comparable to the pattern of change in fetal movement counts reported by Sadovsky et al.[11]

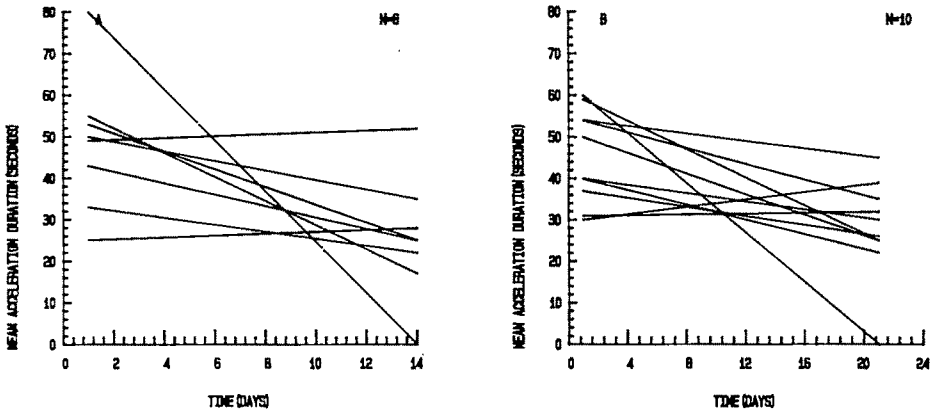


Fig. 3. *Panels A* and *B* show the trends in mean baseline fetal heart rates in sequential nonstress tests of fetuses with abnormal outcomes, which were studied over 2-week and 3-week intervals, respectively. Four of the 18 fetuses had significant elevation of their baseline heart rate exceeding 155 bpm (runs tests, $p < 0.01$).

Late or variable decelerations present (last test)	*Complications*	*Delivery mode†*
No	Intrauterine growth retardation	CS
No	Intrapartum fetal distress, Apgar score <7	VD
No	Intrapartum fetal distress, Apgar score <7	CS
No	Acidemia, Apgar score <7	CS
No	Intrauterine growth retardation	CS
Yes	Intrauterine growth retardation	VD
Yes	Intrapartum fetal distress	CS
No	Postmaturity, intrapartum fetal distress, Apgar score <7	CS
No	Intrapartum fetal distress	CS
No	Intrapartum fetal distress, intrauterine growth retardation, Apgar score <7	CS
No	Intrauterine growth retardation, Apgar score <7	CS
Yes	Intrapartum fetal distress	CS
No	Intrauterine growth retardation	VD
Yes	Intrapartum fetal distress	VD
Yes	Intrauterine growth retardation	VD
Yes	Intrauterine growth retardation	VD
No	Intrauterine growth retardation, Apgar score <7	CS
Yes	Anemia, respiratory distress syndrome	CS

Furthermore, these changes in nonstress test patterns preceded other signs of fetal compromise, for example, late or severe variable decelerations, which were not evident until the last tracing before delivery. As subsequent contraction stress tests were not performed on all patients, it is not possible to determine whether these decelerations would have appeared sooner. Therefore the issues of which FHR alterations provide the earliest sign of fetal compromise or whether contraction stress tests are a more sensitive primary test[13] cannot be conclusively resolved by this study. These data however, do indicate that sequential declines in movement-associated FHR acceleration frequency which exceed 50% or falls in movement-associated FHR acceleration duration which exceed 40% should prompt the performance of a contraction stress test even if the nonstress tests themselves meet normal reactivity criteria.

Criteria for nonstress test interpretation which rely solely on minimum counts of accelerations result in relatively low test sensitivity.[14] The present study supports that observation and the hypothesis that observation of additional nonstress test parameters and their serial trends might improve test sensitivity. Detection of serial changes in these nonstress test parameters may require more frequent testing, that is, every 2 to 4 days, and more careful attention to the details of FHR patterns than has been traditionally advocated. Sequential comparisons of nonstress tests may lead to earlier recognition of potentially compromised fetuses and to more timely initiation of other diagnostic tests such as the contraction stress test. These considerations are most important to clinicians who currently rely on nonstress testing as their first line of antepartum fetal assessment.

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Cesarean delivery of the second twin after vaginal delivery of the first twin

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Cesarean birth of twin B after the vaginal delivery of twin A is an infrequent event. There is little information in the literature documenting outcome of vaginal-abdominal delivery. We reviewed our 10-year experience in the management of such cases and compared outcomes with twins delivered by repeat cesarean section and vaginal delivery in the same time period. Twenty-one cases of combined vaginal–cesarean section deliveries were managed at our institute in a 10-year period. There was no apparent increase in maternal and perinatal morbidity and mortality when twin B was delivered by cesarean section after vaginal delivery of twin A. (AM J OBSTET GYNECOL 1986;154:936-40.)

Key words: Twins, cesarean section, vaginal delivery

The increase in perinatal morbidity and mortality in twin gestation is well recognized.[1, 2] Most authors[3-5] have stressed the increased risk to the second twin. One subset of second twins, those delivered by cesarean section after vaginal delivery of twin A, have gained little attention. Earlier reports of cesarean section done for the second twin were mainly in the form of case reports.[6] Recently, Evrard and Gold[7] in reporting four cases during a 4-year period were able to add only five cases from the recent literature. Although the maternal and perinatal mortality and morbidity with this mode of delivery have been assumed to be high,[8] unless cesarean section is used for all twins, this infrequent complication cannot always be avoided.

In an attempt to define the risks of cesarean section delivery of twin B after vaginal delivery of twin A, we selected such cases from a retrospective review of our last 10 years' experience with multiple gestation.

Material and methods

All twin deliveries performed at Tampa General Hospital from January 1, 1973, to December 31, 1982, were reviewed. Twin deliveries wherein the firstborn twin was delivered vaginally and a cesarean section was done for twin B (combination delivery) were selected for this report.

Factors selected for analysis were the incidence of combination delivery during the study period, the indication for combination delivery, interval between de-

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Table I. Cesarean section in multifetal gestations, 1973 to 1982

Year	Total deliveries	Total cesarean section rates (%)	Twin pregnancies (n)	Cesarean section in twins		Cesarean section for twin B	
				n	%	n	%*
1973-1974	6,889	7.8	61	10	16	1	10
1975-1976	5,997	8.5	58	13	22	1	8
1977-1978	6,221	11.1	74	27	36	8	29
1979-1980	7,190	13.	77	34	44	4	11
1981-1982	8,014	15.7	82	51	62	7	13
Total	34,411		352	135		21	

*Percent of Total cesarean section rate in twins.

Table II. Primary indication for cesarean section in twin B

Indication	No. of cases	%
Transverse lie	7	33
Fetal distress	5	24
Contracted cervix	4	20
Prolapsed cord	2	9
Premature breech	2	9
Failed extraction	1	5
Total	21	

Table III. Delivery interval and Apgar scores of second twins

Time interval (min)	No. of twins	5 min Apgar scores <7 (No.)
0-10	0	—
10-20	7	2
21-60	9	0
61-120	5	1
Total	21	3

liveries, Apgar scores, intrapartum fetal and neonatal deaths, duration of maternal hospital stay, and maternal febrile morbidity.

Results

During the study period there were 352 twin deliveries and 21 (6%) of these were combination deliveries that totaled 15% of the 135 cesarean births for twin gestation. Although the overall rate of cesarean births for twin deliveries rose from 16% in 1973 and 1974 to 62% in 1981 and 1982, the percentage of combination deliveries among cesarean births in twin gestation varied between 8% and 29% with no apparent trend (Table I). Indications for cesarean birth of twin B are shown in Table II. Persistent transverse lie was the most common indication followed by fetal distress. Prolonged delivery interval (approximately 1 hour or more) associated with a contracted cervix was the indication for cesarean section in 20% of the cases.

Approximately half of the cases of combination delivery occurred at or beyond 35 weeks of gestational age (52%). Four gestations (19%) were between 33 and 36 weeks and six (29%) were between 29 and 33 weeks. The mean difference in weight between twins was 193 gm with a range of 0 to 1195 gm. A weight difference of >250 gm was seen in only four cases. In three, twin A weighed more than twin B, and in one, twin B weighed more than twin A. Seven infants weighed <1500 gm (17%), 28 (66%) weighed between 1500 and 2500 gm, and seven (17%) weighed >2500 gm.

The mean interval between deliveries was 38 minutes with a range of 12 to 115 minutes. The interdelivery interval did not appear to influence neonatal condition as reflected by the distribution of low Apgar scores (Table III). One-minute Apgar scores of <7 were more common in twin B, but 5-minute Apgar scores were similar between the two groups. Two of the three infants with low 5-minute Apgar scores in the cesarean birth group were delivered for intrapartum fetal distress after the delivery of twin A. There were two perinatal deaths (47 per 1000), both of which occurred in the twins delivered vaginally. The stillbirth was an intrapartum fetal death during maternal transport for preterm labor. Twin B was delivered by cesarean section because of fetal distress. The neonatal death occurred in a newborn infant at 30 weeks of gestation after prolonged rupture of the membranes. Neonatal cultures revealed group B streptococcal sepsis. Twin B, delivered by cesarean section because of breech presentation, had an uneventful neonatal course without evidence of infection.

A comparison of birth weights and Apgar scores of all twins delivered during the study period (at this institution) were grouped according to method of delivery (Table IV). The comparisons between groups revealed that the only significant difference was in the 1-minute Apgar score for twin B. Second twins delivered by repeat cesarean section had higher 1-minute Apgar scores than those delivered vaginally or by cesarean section after a vaginal delivery of twin A. The 5-minute Apgar scores for these groups, however, did not differ statistically.

Table IV. Birth weights and Apgar scores according to method of delivery

Group	n	Twin A			Twin B		
		Weight (gm)	*1 min Apgar score*	*5 min Apgar score*	*Weight (gm)*	*1 min Apgar score*	*5 min Apgar score*
Repeat cesarean section	18	2207	7.43	8.64	2164	7.85*	8.78
Primary cesarean section	115	2188	7.07	8.49	2134	6.64	8.39
Combination	21	1987	7.04	8.13	1999	5.52*	7.95
Vaginal	198	2153	6.83	8.23	2144	6.20*	7.87

*$p<0.05$, Duncan's multiple range test; difference between repeat cesarean section versus combination and repeat cesarean section versus vaginal delivery.

All mothers received intraoperative antibiotic prophylaxis. During this 10-year period, although prophylactic regimens varied between triple antibiotic therapy in earlier years to single antibiotic prophylaxis in recent years, the regimen used did not appear to influence the outcome. Postpartum febrile morbidity occurred in six mothers (28%) but significant febrile morbidity leading to prolonged hospital stay was seen in only three mothers (14%). In the patients with prolonged hospital stay, the following risk factors were present: severe anemia, severe preeclampsia, and intrauterine manipulation in a case of failed breech extraction. Overall, hospital stay varied between 5 and 13 days with a mean of 6.7 days and a median of 6 days.

Comment

In an effort to reduce perinatal mortality and morbidity, use of cesarean section as a method of choice for delivery of twins has been advised if one or both twins presents as a breech or is in a transverse lie.[9, 10] When vaginal delivery is planned, vaginal delivery of twin B is anticipated after twin A has been delivered. At times, however, this becomes difficult or unwise. Because of the paucity of information regarding this subject, physicians may be reluctant to perform a cesarean birth for twin B when twin A is delivered vaginally. Infallible diagnosis before delivery and cesarean section for all twin pregnancies would be required to avoid this infrequent possibility. We found that 21 of the 352 twins delivered (6%) required cesarean section after vaginal delivery of twin A, totaling 15% of all cesarean births (n = 135) in twin pregnancies. Even with liberal use of cesarean birth since 1979 (Table I), 11 of the 85 cesarean sections done in twins were for twin B and they formed 13% of all cesarean births in twin pregnancies.

In up to 40% of cases, both twins may present by the vertex[11] and would therefore be candidates for vaginal delivery, according to most recommendations. However, failure to diagnose twins before the onset of labor could be expected to contribute to the need for cesarean section of the second twin. In our review lack of diagnosis before labor occurred in 35% of all cases. Additionally, unexpected low birth weight may become apparent only after delivery of the first twin and cesarean section has been proposed as an optimal route of delivery for twin B.[12]

In their recent review of this subject, Evrard and Gold[7] had no cases where the indication for cesarean section was prematurity, cord prolapse, or fetal distress. Both perinatal deaths, moreover, occurred in the twin delivered by cesarean section. In our experience both perinatal deaths were in the twin delivered vaginally. This difference in experience may be due to the limited sample size of the series or may reflect differing methods of management of twin B.

Two important observations from this series should be stressed. First, even with liberalized indications, the necessity for cesarean section may not always be recognized before delivery. Second, in this small series there appeared to be little correlation between interdelivery interval and outcome; however, a larger series is required to support definitive correlations between interdelivery intervals and outcomes.

The need for adequate antepartum diagnosis for optimal management of twin gestation is obvious and has been stressed by various authors.[13, 11] In view of the continued problem of inadequate prenatal care and the cost efficacy of routine sonographic examination in all patients, however, the problem of undiagnosed twins is likely to remain with us. As noted in this series, moreover, even with optimal conditions for vaginal delivery, intrapartum events may alter the management of twin B.

The feasibility of such an alteration was demonstrated in the outcome of twin B. Although an inter–twin delivery time of 15 to 20 minutes has been suggested as a limit of safety, there appeared to be no great increase in compromised infants delivered by cesarean section at intervals up to 2 hours. Beginning in 1979, all second twins were evaluated with a fetal monitor, and after delivery of twin A a fetal electrode was placed as soon as it was found safe to do so. The overall outcome of newborn infants delivered in such a manner,

moreover, was not significantly different from that of those delivered vaginally.

In most reports of post–cesarean section morbidity, infection has been described as the most important complication, ranging from 19% to 51%.[15, 16] In our review, significant febrile morbidity leading to prolonged hospital stay was seen in three of 21 patients (14%). All had predisposing factors such as severe anemia, severe preeclampsia, or intrauterine manipulation.

This review, which forms the largest number of such cases reported, suggests that whenever indications exist, cesarean section can be performed safely for the delivery of twin B after vaginal delivery of twin A. We recognize that indications for cesarean section are controversial; however, in this series there was no excessive increase in maternal morbidity and neonatal morbidity or mortality due to cesarean birth for twin B. We therefore feel that cesarean birth of twin B after vaginal delivery of twin A is a safe and acceptable method of management in a selected group of twin deliveries, but we do not condone undue hesitation in clinical decision making.

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Lactation suppression and puerperal fever

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Seventy-five puerperal women who did not wish to breast-feed were treated with bromocriptine mesylate (Parlodel) or placebo in a prospective, randomized, double-blind study. Results revealed a puerperal fever incidence of 18.6%, 13.3% of which was due to breast engorgement and 5.3% to an infectious process. Parlodel prevented puerperal fever in 87.9% of patients. If Parlodel was given within 18 hours of delivery, physiologic puerperal fever was prevented in 100% of cases. (Am J Obstet Gynecol 1986;154:940-1.)

Key words: Lactation, breast engorgement, puerperal fever, milk fever, bromocriptine mesylate (Parlodel)

The purpose of this study is to prove existence of puerperal fever due to breast engorgement and that prevention of this fever can be achieved by lactation suppression.

Material and methods

A prospective, randomized, double-blind study was conducted with 92 puerperal patients at the University of South Alabama Medical Center from January to May, 1985. Healthy patients were recruited from the obstetrics service according to the following criteria: (1) Patients were in the immediate puerperium, having had an uncomplicated pregnancy; they had no medical illnesses, were afebrile in the intrapartum period, and were delivered vaginally. (2) Selection was without regard to age, race, or parity. (3) Patients did not intend to breast-feed and agreed to suppress postpartum lactation by pharmacologic means. (4) Patients had gestational ages ≥36 weeks at delivery. (5) Patients remained hospitalized for 72 hours in order to allow collection of sufficient objective data. Patients were supplied medicine bottles in routine fashion after vital signs stabilized. Forty-five patients received Parlodel, and 47 received a placebo. Tablets from unknown numbered bottles were dispensed until postpartum day 3 (day of discharge), at which time patients were given Parlodel for 2 weeks. Before discharge, a fasting 7 ml blood sample was obtained from the antecubital space, spun down, and sent for assay of serum calcium.

Puerperal fever was defined as one or more oral readings of ≥100° F (37.8° C). Vital signs were recorded every 4 hours. Patients were instructed to notify the nurse when breast engorgement began to document time. Febrile episodes were investigated by physical examination, complete blood count with differential count, cervical culture, microscopic analysis, and culture of a catheterized urine specimen. Fever was considered due to physiologic breast engorgement if four postulates were satisfied: (1) fever of a transient nature, (2) appearance of a temperature spike within 2 hours of onset of breast engorgement and lactation, (3) no other signs or symptoms (including laboratory studies) present suggesting an infectious process, (4) resolution of fever without use of antibiotics.

Table I. Summary of puerperal fevers

	Bromocryptine mesylate (Parlodel)		*Placebo*	
	n	%	*n*	%
Febrile	0	0.0	6	9.0
Afebrile	32	47.8	29	43.2

Fisher's exact test (n = 67 patients; no infectious source; received bromocriptine mesylate or placebo within 18 hours post partum): p = 0.03.

Eight patients were excluded: Four had an obvious infectious cause of puerperal fever, and four received Parlodel more than 18 hours post partum.

Results

Seventy-five patients satisfied study criteria. Seventeen patients excluded were discharged early (insufficient objective data collection) or because of errors in taking medication. Fourteen of 75 patients had puerperal fever (18.6%). Ten (13.3%) were considered lactation-associated. Three febrile patients had upper respiratory infections and one developed endometritis with cultured β-hemolytic streptococci and staphylococci. Four of 10 febrile patients with breast engorgement were treated with Parlodel, and the remaining six received placebo. It was statistically significant that if the time from delivery to administration of Parlodel

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Table II. Summary of puerperal fever in the study population

	Total patients	% of total population	% of total fevers	Oral temperature (°F)	
				Range	Mean
Physiologic breast engorgement and lactation	10	13.3	71.4	100.0-101.2	100.4
Upper respiratory infection	3	4.0	21.4	100.0-101.0	100.5
Endometritis	1	1.3	7.1	102.8	
All causes	14	18.6	–	100.0-102.8	100.8

was <18 hours, no patient manifested physiologic puerperal fever (Table I). Puerperal fevers are summarized in Table II. Serum calcium levels were subnormal or low normal in 95% of patients regardless of medication, placebo, or fever.

Comment

The double-blind study demonstrates that milk fever is a true physiologic entity, and suppression of lactation with Parlodel, if given within 18 hours of delivery, eliminates this fever.

At least one study and several texts unequivocally deny puerperal fever associated with breast engorgement despite Meigs' elegant description more than 130 years ago. Roser[1] suggested empirical antibiotic therapy could be withheld to avoid an iatrogenically complicated puerperium, and suppression of lactation would eliminate the fever. Veterinary medicine has long recognized the devastating effects of milk fever in cattle, such as hypocalcemia, changes in neuromuscular tone, tremor, paresis, coma, and death.[2]

Puerperal fever in the past was undoubtedly attributed to genitourinary tract infection. At present it is still caused by cystitis, endometritis, atelectasis, and pelvic phlebitis. Results of this study show that puerperal fever among women with a benign intrapartum course may be caused by physiologic breast engorgement, and Roser's suggestion of eliminating empirical antibiotics is valid.

Bromocriptine mesylate (Parlodel) and placebos were supplied by the Pharmaceutical Division, Sandoz Inc., East Hanover, New Jersey.

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Atraumatic delivery in cases of malpresentation of the very low birth weight fetus at cesarean section: The splint technique

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A technique to assist in the atraumatic delivery of the very low birth weight fetus in cases of malpresentation at cesarean section is described; the technique minimizes potential trauma associated with version and/or extraction techniques. Use of uterine relaxing anesthetic agents and subsequent risk of hemorrhage are avoided. (Am J Obstet Gynecol 1986;154:941-2.)

Key words: Premature fetus, malpresentations, atraumatic cesarean delivery

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When delivery is inevitable, current management of the very low birth weight fetus (501 to 1500 gm, 26 to 32 weeks' gestation) consists of atraumatic delivery and intensive neonatal care. Such management has led to increased survival rates and decreased morbidity for these neonates.

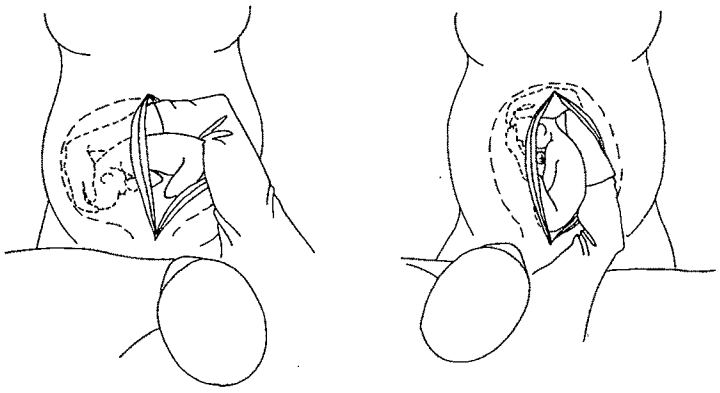


Fig. 1. The splint technique.

Cesarean section has been liberally used in an attempt to decrease intrapartum hypoxia and birth trauma. Mode of delivery of the fetus in cephalic presentation is controversial. Some advocate elective cesarean section of all very low birth weight fetuses while others believe that continuous electronic fetal heart rate monitoring allows early identification of the stressed fetus.[1] There is less controversy regarding the route of delivery in cases of malpresentation. Many authorities agree that cesarean section is indicated in such cases.[2]

A poorly developed lower uterine segment often makes a vertical uterine incision necessary (either low vertical, confined to the lower uterine segment, or classical, with the incision carried up to the uterine fundus). In spite of adequate uterine incision, difficulty in delivering the very low birth weight fetus presenting in a transverse lie or as a breech is sometimes encountered. Internal podalic version and breech extractions may be traumatic, even at cesarean section.[3] The very low birth weight fetus is particularly vulnerable to manipulative procedures, and excessive bruising may lead to significant blood loss and jaundice.[4] A technique that minimizes potential trauma with version and/or extraction techniques is described.

The technique consists of "splinting" the entire fetus, with the head held in the operator's hand, the trunk flat against the forearm, and the buttocks stabilized in the antecubital fossa (Fig. 1). In cases of a transverse lie, the entire fetus can be turned to either a cephalic or a breech presentation with subsequent atraumatic delivery. In my experience, the majority of these fetuses can be brought down as vertex presentations. Those who are brought down as a breech presentation or those who are de novo in breech presentations can be delivered up to the umbilicus, following which assisted breech delivery is performed. In many of my cases, however, the entire fetus was delivered while "splinted." Uterine relaxation with anesthetic agents was never required to accomplish the delivery. The technique has been used successfully in 25 cases.

The adult upper limb is uniquely suited for this type of technique, as the measurements of the hand and forearm correspond well to the measurements of the very low birth weight fetus. Fetal head circumference ranges from approximately 25 cm at 28 weeks' gestation to approximately 30 cm at 32 weeks' gestation. The crown-rump length of a fetus varies from 23 cm at 26 weeks' gestation to 28 cm at 32 weeks' gestation. In the author's case, the distance from the third terminal digit to the wrist is 19 cm, and that from the wrist to the antecubital fossa is 26 cm.

In summary, a technique to assist in the atraumatic delivery of the very low birth weight fetus at the time of cesarean section has been described which is applicable in cases of fetal malpresentation, either breech or transverse lie. A vertical uterine incision is often necessary. Intact amniotic membranes are helpful but not essential. Use of uterine relaxing anesthetic agents and subsequent risk of hemorrhage are avoided. If the technique fails, traditional management of uterine relaxation and version/extraction remains an option.

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Cytosol estrogen receptor content of female parietal peritoneum

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The ovarian and peritoneal mesothelium are believed to derive from the same embryonal coelomic epithelium. Neoplastic proliferation of these epithelia is sometimes grossly and histologically similar. Recently estrogen receptors have been identified in gynecologic neoplasms. With the use of standard techniques, the parietal peritoneum was assessed for estrogen receptor content in 17 women undergoing celiotomy for various reasons. All peritoneal specimens were negative for estrogen receptor content. This suggests a heterogeneous origin of the peritoneal surface epithelium and that peritoneum may not be müllerian in origin. (AM J OBSTET GYNECOL 1986;154:943-4.)

Key words: Estrogen receptors, parietal peritoneum

The mesothelium of the ovary and of the peritoneal cavity is believed to be the product of the same embryonal coelomic epithelium.[1] Peritoneal mesothelium may respond as ovarian mesothelium when appropriately stimulated.[2] Both benign and malignan gynecologic neoplasms contain estrogen receptor proteins. The purpose of this study was to determine whether estrogen receptor proteins are present in parietal peritoneum.

Material and methods

A 1 by 1 cm portion of normal parietal peritoneum was obtained in 17 women with various gynecologic pathologic conditions at celiotomy (Table I). The excised tissue was placed on dry ice and maintained at −20° C until assayed. For assay, the tissue was homogenized in a buffer consisting of 0.01 mol/L Tris, 1.5 mol/L sodium ethylenediaminetetraacetic acid, and 5 mmol/L dithiothreitol, pH 8.0, at 2° C. Cytosol was prepared as the 30-minute, 350,000 × g supernatant fraction of the homogenate. Cytosol estroger receptors were measured by saturation binding analysis, with a range of 0.2 to 5 nmol/L for radiolabeled estradiol. Radiolabeled estradiol was obtained from New England Nuclear, Boston, Massachusetts [(2,4 6,7-^{3}H) 17β-estradiol, 100 Ci/mmol]. It was purified before use by successive descending paper chromatography in two separate solvent systems. At each dosage, a duplicate assay was performed in the presence of a respective 100-fold molar excess of unlabeled 17β-estradiol to allow correction for nonspecific binding. Samples were incubated for 18 hours at 4° C to achieve binding equilibrium. Protamine sulfate precipitation was used to separate free from receptor-bound steroid. Specific binding was calculated as the difference between total and nonspecific values at each steroid level. The data were examined graphically by direct linear plot analysis for determination of the receptor-binding affinity and capacity of the tissue.

Results

The results are given in Table I. As shown, all specimens analyzed for cytosol estrogen receptor content were negative. In the five patients with serous cystadenocarcinoma, three were moderately differentiated and two were undifferentiated. The degree of tumor differentiation was not associated with the presence of estrogen receptors in normal uninvolved parietal peritoneum.

Comment

Our finding of no estrogen receptors in biopsy specimens of normal parietal peritoneum suggests that the parietal peritoneum may not be of müllerian origin.

Many undifferentiated ovarian cancers may not be gonadal in origin but rather are malignant mesotheliomas. These may arise primarily as tumors of the peritoneal surface, only secondarily involving the ovary.[2] In addition, endometriosis is believed to represent, for the most part, a metaplastic alteration of pelvic mesothelium mediated through sex steroid receptor proteins responsive to the endocrine milieu. It appears, therefore, that there is multipotentiality for

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Table I. Summary of patient data

No. of patients	*Age (yr)*	*Pathologic diagnosis*	*Cytosol estrogen receptor content of normal peritoneum*
5	57-82	Stage III serous cystadenocarcinoma	Negative
1	36	Stage IB squamous cell carcinoma of cervix	Negative
1	27	Bilateral benign cystic teratoma	Negative
4	26-34	Endometriosis	Negative
1	50	Endometrial hyperplasia	Negative
5	34-54	Uterine leiomyoma	Negative

differentiation of müllerian oriented epithelium conditioned by embryonic origin.[1] This view may explain the occurrence of peritoneal tumor involvement without an obvious primary site of origin. This view, however, must be seriously questioned in light of our finding of no estrogen receptors in normal parietal peritoneum.

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SYMPOSIUM

Symposium on Cefotetan

The following papers are selected from presentations given at an "Investigator's Symposium on Cefotetan," held on September 12-15, 1985.

Supported by an educational grant from Stuart Pharmaceuticals, Wilmington, Delaware.

Introduction

Those of us in the fields of infectious disease and surgery are now in the enviable position of having not a drug of choice but drugs of choice for prophylactic and therapeutic use against infection. A wide variety of antimicrobial agents is available with established efficacy and safety. Though they are not identical in their activity against various pathogens, none stands clearly above the others in terms of clinical usefulness.

As the papers presented at this symposium demonstrate, cefotetan, a new broad-spectrum, long-acting parenteral cephalosporin, can now be added to the array of effective agents. Cefotetan has microbiologic activity very similar to that of cefoxitin, inhibiting most Enterobacteriaceae, gram-positive cocci (except the Enterococcus), and anaerobes, including the troublesome *Bacteroides fragilis*. Cefotetan is as effective as cefoxitin in the settings described here and can be expected to be very useful in community-acquired intra-abdominal sepsis, propylaxis for colorectal, obstetric, and gynecologic procedures, abdominal trauma, and other settings.

In the current climate of health care, with roughly equivalent antibiotic agents, cost becomes an important issue. Because of its long half-life, cefotetan can be given twice per day in situations where other agents must be given three or four times per day. The cost savings that will result from a reduction in the number of administrations per day and the demands on nursing and pharmacy staff could be substantial. Therefore, based on my own research and that presented in this symposium, I expect that cefotetan will find an important role in prophylaxis and treatment of infection in situations where a broad spectrum antibiotic is needed.

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Cefotetan in the treatment of obstetric and gynecologic infections

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The efficacy, tolerance, and safety of cefotetan—a new 7-α-methoxy cephalosporin—was assessed in controlled and uncontrolled evaluations involving 131 evaluable patients hospitalized with obstetric and gynecologic infections. The 99% satisfactory clinical response rate obtained with this drug was equivalent to that obtained with either moxalactam or cefoxitin, yet the mean amount of cefotetan given was lower than that of the other two drugs. Cefotetan was well tolerated and produced no major adverse reactions. In this era of Diagnosis Related Groups and cost containment, the twice-daily dosage schedule of cefotetan is a decided cost benefit. (AM J OBSTET GYNECOL 1986;154:946-50.)

Key words: Cefotetan, endometritis, pelvic inflammatory disease, antibiotics

In treating obstetric and gynecologic infections, an antibiotic with a broad spectrum of antibacterial activity and resistance to β-lactamase-producing species is often crucial to adequate patient response. Cefotetan—a new semisynthetic parenteral cephalosporin derivative and β-lactam antibiotic—has high resistance to hydrolysis by Richmond-Sykes types I, III, and IV β-lactamases of gram-negative bacteria.[1] This resistance is provided by the 7-α-methoxy group. The stability of cefotetan to β-lactamase is as high as that of cefoxitin; the two antibiotics have similar chemical structures.

Another notable feature of cefotetan is an elimination half-life of >3 hours, which is longer than that of most commercially available cephalosporins. Normal volunteers had high plasma levels of cefotetan after receiving either 500 mg or 1000 mg every 12 hours.[2] Its relatively long half-life and good antimicrobial activity in treating gynecologic infections—frequently involving polymicrobial combinations of anaerobes and aerobes—make cefotetan an ideal antibiotic for hospitalized patients requiring more than 48 hours of intravenous treatment. Twice-daily administration of a single agent such as cefotetan is of decided advantage in this era of Diagnosis Related Groups, prospective payment schedules, and cost containment.

In an in vitro study involving 1063 gram-negative isolates, concentrations of cefotetan ranging from 0.007 to 3.2 mg/L inhibited 90% of all isolates of *Escherichia coli, Klebsiella* and multiresistant strains of this organism, *Proteus mirabilis, P. vulgaris, Providencia rettgeri, P. stuartii, Serratia marcescens,* and *Citrobacter freundii.*[3] Cefotetan is as active as moxalactam, a related cephalosporin, against gram-positive cocci. Cefotetan has moderate activity against *Staphylococcus aureus* and *Streptococcus pyogenes.*[3]

In another in vitro study involving 12,260 isolates, the inhibitory spectrum of cefotetan paralleled the spectra of the newest generaton of cephamycins and exceeded those of cefoxitin and cefamandole.[1] Cefotetan has high activity against *Neisseric gonorrhoeae.*[4]

All studies have shown that cefotetan is either inactive or has very poor activity against *Pseudomonas aeruginosa, Acinetobacter* organisms, enterococci, and methicillin-resistant *S. aureus.* Cefotetan is active against *Serratia* and *Providencia* organisms and Enterobacteriaceae—three of the problem organisms of hospitals.

To obtain additional evidence on the efficacy, tolerance, and safety of twice-daily intravenously administered cefotetan in treating obstetric and gynecologic infections, controlled and uncontrolled studies were conducted with cefotetan.

Materal and methods

The study protocol was reviewed and approved by the Institutional Review Board for Experiments Involving Human Subjects. Written informed consent was obtained from all patients.

Hospitalized patients originally seen in the emergency room for complaints related to acute pelvic infection were entered into the studies. These patients had either known or suspected bacterial disease and were considered likely to meet all study criteria. No patient was entered into evaluation who was pregnant or breast-feeding, had a history of penicillin allergy, or received antibiotics immediately before hospital admission. The study populations were primarily young and nonwhite; many of the patients had underlying

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Table I. Characteristics of study subjects

	Controlled studies			*Uncontrolled study of cefotetan*	*Overall cefotetan studies*
	Cefotetan	*Moxalactam*	*Cefoxitin*		
Number of evaluable cases	34	8	5	84	118
Age distribution (yr)					
<20	8	1	1	22	30
20-39	24	7	4	61	85
40-59	2	0	0	1	3
Mean	25.4	22.8	24.4	24.3	24.6
Other underlying conditions					
None	7	0	1	29	36
Bronchopulmonary	2	0	1	1	3
Cardiovascular	3	1	0	7	10
Hematologic	2	1	0	18	20
Hepatobiliary	0	0	0	1	1
Genitourinary	11	4	0	8	19
Chronic addiction	5	0	0	8	13
Postoperative	6	4	4	48	54
Posttrauma	1	1	0	0	1
Other	4	3	0	5	9

Table II. Types of infection and treatment

	Controlled studies			*Uncontrolled study of cefotetan*	*Overall cefotetan studies*
	Cefotetan	*Moxalactam*	*Cefoxitin*		
Number of evaluable cases	34	8	5	84	118
Diagnosis					
Endometritis	11	2	4	48	59
Pelvic inflammatory disease	19	5	1	33	52
Salpingitis	2	1	0	0	2
Other	2	0	0	3	5
Source of infection					
Community-acquired	26	7	1	35	61
Hospital-acquired	8	0	4	45	53
Unknown	0	1	0	4	4
Dosage schedule					
2.0 gm every 12 hr	34	0	0	54	88
3.0 gm every 12 hr	0	0	0	30	30
2.0 gm every 8 hr	0	8	4	0	0
1.0 gm every 8 hr	0	0	1	0	0
Duration of therapy (days)					
2-4	0	0	0	1	1
5-10	32	8	5	82	114
11-16	2	0	0	1	3
Mean	5.9	5.8	7.2	5.9	5.9
Total amount of drug given (gm)					
<10	0	0	0	1	1
10-19	7	0	1	8	15
20-29	25	1	0	45	70
30-39	0	7	2	30	30
40+	2	0	2	0	2
Mean	21.5	31	36	25.2	24.1

diseases or conditions in addition to their pelvic infections (Table I).

All infections were confirmed by clinical findings and by isolation of pathogens and their identification as to genus and species. Cultures of intrauterine sites were obtained by a sterile double-lumen transcervical catheter.[5] Urine and blood cultures were also obtained. Pathogen susceptibility to cefotetan was determined by disk testing with the Kirby-Bauer method or miminal inhibitory concentration determination, or with both.

By random assignment, patients received either cefotetan, moxalactam, or cefoxitin. Additional patients were entered into a separate uncontrolled evaluation with use of cefotetan. During the treatment period the study subjects received no other antimicrobials.

Saftey of cefotetan treatment was assessed by comparing pretherapy and posttherapy hematologic, renal,

Table III. Overall clinical response in controlled and uncontrolled studies

Diagnosis	*Cefotetan-treated patients*			*Moxalactam-treated patients*			*Cefoxitin-treated patients*		
		Clinical response			*Clinical response*			*Clinical response*	
	No. of patients	*Satisfactory*	*Unsatisfactory*	*No. of patients*	*Satisfactory*	*Unsatisfactory*	*No. of patients*	*Satisfactory*	*Unsatisfactory*
Endometritis	59	59	0	2	2	0	4	4	0
Pelvic inflammatory disease	52	51	1	5	5	0	1	1	0
Salpingitis	2	2	0	1	1	0	0	0	0
Other	5	5	0	0	0	0	0	0	0
Total	118	117 (99%)	1 (1%)	8	8 (100%)	0	5	5 (100%)	0

Table IV. Clinical response in controlled studies

Diagnosis	*Cefotetan-treated patients*			*Moxalactam-treated patients*			*Cefoxitin-treated patients*		
		Clinical response			*Clinical response*			*Clinical response*	
	No. of patients	*Satisfactory*	*Unsatisfactory*	*No. of patients*	*Satisfactory*	*Unsatisfactory*	*No. of patients*	*Satisfactory*	*Unsatisfactory*
Endometritis	11	11	0	2	2	0	4	4	0
Pelvic inflammatory disease	19	19	0	5	5	0	1	1	0
Salpingitis	2	2	0	1	1	0	0	0	0
Other	2	2	0	0	0	0	0	0	0
Total	34	34 (100%)	0	8	8 (100%)	0	5	5 (100%)	0

Table V. Clinical response of cefotetan-treated patients in uncontrolled studies

Diagnosis	*Cefotetan-treated patients*		
		Clinical response	
	No. of patients	*Satisfactory*	*Unsatisfactory*
Endometritis	48	48	0
Pelvic inflammatory disease	33	32	1
Salpingitis	0	0	0
Other	3	3	0
Total	84	83 (99%)	1 (1%)

and hepatic parameters. When treatment was prolonged, laboratory tests of these parameters were repeated at least weekly. Follow-up was made when these test results were abnormal. Patients were assessed daily for local tolerance to the intravenous injections and for adverse reactions to the drug. The pharmacokinetics of cefotetan were evaluated by determining peak and trough serum levels and urine concentrations, with use of plasma and urine specimens obtained on day 2, 3, or 4 of the study.

Both clinical and bacteriologic responses to treatment were assessed. Clinical response was considered satisfactory if local or systemic signs of infection had disappeared or were substantially improved after treatment. Bacteriologic response was determined by comparing isolates obtained before and after therapy and was considered satisfactory if causative pathogens were eradicated or if a good clinical response was obtained despite the presence of a new pathogen.

Laboratory data were analyzed by linear regression coefficients and slopes, with use of drug therapy as the independent variable. Missing patient data were eliminated from statistical analysis.

Results

A total of 118 recipients of cefotetan, 34 in the controlled study and 84 in the uncontrolled study, were judged evaluable for efficacy, while all recipients of this drug were used in the analyses of laboratory values and adverse effects. The pelvic infections of evaluable cefotetan recipients were diagnosed as endometritis (59 cases), pelvic inflammatory disease (52 cases), salpingitis (two cases), or "other" (five cases). Sixty-one evaluable cefotetan recipients had community-acquired infections; 53, hospital-acquired infections; 4, infections of unknown source (Table II).

In the controlled study, 34 evaluable patients received 2 gm of cefotetan every 12 hours; eight patients, 2 gm of moxalactam every 8 hours; four patients, 2 gm of cefoxitin every 8 hours; and one patient, 1 gm of cefoxitin every 8 hours. In the uncontrolled study of cefotetan, 54 evaluable patients received 2 gm every 12 hours, and 30 evaluable patients received 3 gm every 12 hours. Of the overall 118 evaluable recipients of cefotetan, 88 received 2 gm every 12 hours and 30 received 3 gm every 12 hours. In the controlled eval-

Table VI. Bacteriologic response by pathogen to cefotetan

Pathogen	No. of isolates			Bacteriologic response	
	Single organism infection	Polymicrobial infection	Total	Satisfactory*	Unsatisfactory
Staphylococcus aureus	2	7	9	9	0
S. epidermidis	1	16	17	17	0
Streptococcus pyogenes	3	2	5	5	0
S. agalactiae	6	17	23	22	1
Streptococcus sp	7	27	34	32	2
Enterococcus	0	7	7	7	0
Corynebacterium sp	0	1	1	1	0
Escherichia coli	5	19	24	23	1
Klebsiella pneumoniae	2	3	5	4	1
Enterobacter sp	0	5	5	5	0
Proteus mirabilis	2	8	10	10	0
Citrobacter sp	2	0	2	2	0
Haemophilus sp	2	7	9	8	1
Neisseria gonorrhoeae	22	17	39	38	1
Acinetobacter var *anitratus*	0	1	1	1	0
Bacteroides fragilis	0	2	2	2	0
B. melaninogenicus	1	6	7	7	0
B. bivius	0	8	8	7	1
Bacteroides sp	0	11	11	9	2
Fusobacterium sp	3	4	7	7	0
Clostridium sp	0	1	1	1	0
Eubacterium sp	0	1	1	1	0
Peptococcus sp	0	5	5	5	0
Peptostreptococcus sp	0	3	3	3	0
Veillonella sp	0	3	3	2	1
Other anaerobes	2	6	8	8	0

*Includes patients in whom there was no material to culture because of clinical improvement.

Table VII. Plasma and urine levels of cefotetan

Dosage schedule	Plasma concentrations (μg/ml)				Urine concentration (μg/ml)	
	Peak		Trough			
	No. of patients	Mean	No. of patients	Mean	No. of patients	Mean
1 gm every 12 hr	1	113.5	1	7.5	1	690.0
2 gm every 12 hr	123	189.8	121	13.5	116	1565.1
3 gm every 12 hr	33	255.6	32	15.5	32	2253.2
Total	157		154		149	

uations the mean durations of therapy were 5.9 days for cefotetan recipients, 5.8 days for moxalactam recipients, and 7.2 days for cefoxitin recipients. The mean duration of cefotetan therapy in the uncontrolled trial was 5.9 days. For the two cefotetan studies combined, the mean duration of treatment was also 5.9 days. In either evaluation of cefotetan, the mean amount of this drug given (21.5 gm in the controlled study, 25.2 gm in the uncontrolled study, 24.1 gm overall) was less than that of moxalactam (31 gm) or cefoxitin (36 gm).

Ninety-nine percent of evaluable cefotetan recipients (n = 118) had a satisfactory clinical response to treatment. The satisfactory clinical response to treatment was 100% for recipients of either moxalactam (n = 8) or cefoxitin (n = 5). There were no statistically significant differences between these clinical response levels (Tables III, IV, and V).

Eleven (4%) of 247 isolates showed an unsatisfactory bacteriologic response to cefotetan therapy (Table VI). Among cefotetan recipients the primary pathogen isolated was *N. gonorrhoeae* (39 isolates). Other isolates of prominence from these patients included *Streptococcus* species (34 isolates), *E. coli* (24 isolates), *S. agalactiae* (23 isolates), and *S. epidermidis* (17 isolates).

Adverse signs and symptoms in cefotetan- or comparator drug–treated patients were infrequent and generally mild in severity. In the controlled protocol, two patients had drug-related adverse signs and symptoms. One cefotetan-treated patient had a report of

monilial vaginitis, and a moxalactam-treated patient had a report of phlebitis. There were 10 reports of drug-related adverse signs and symptoms in six cefotetan-treated patients in the uncontrolled protocol. The 10 reports included serum glutamic-oxaloacetic transaminase increase, serum glutamic-pyruvic transaminase increase, phlebitis, urticaria, rash, papular rash, tachycardia, dyspnea, nausea, and monilial vaginitis.

Plasma and urine assay data are summarized in Table VII.

Comment

These evaluations demonstrated that twice-daily administration of intravenous cefotetan is highly efficacious in treating obstetric and gynecologic infections of hopsitalized patients. Cefotetan was at least as successful as moxalactam and cefoxitin in the comparative evaluations of clinical response. Moreover, the mean amount of cefotetan used was less than that of either moxalactam or cefoxitin—further indicating the high efficacy of cefotetan.

The controlled and uncontrolled evaluations of cefotetan also affirmed the safety of this drug.

We found no statistically significant changes in urinary levels of cefotetan for any of the days measured.

In conclusion, twice-daily administration of intravenous cefotetan is a highly efficacious means of treating obstetric and gynecologic infections of hospitalized patients. The drug, which we found to be safe and well tolerated, can be administered less frequently and at lower doses than can moxalactam and cefoxitin, while still obtaining at least equivalent clinical results.

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Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section

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A study comparing the efficacy of cefotetan versus cefoxitin for prophylaxis in patients undergoing cesarean section was carried out at the University of Iowa. After institutional review, 36 subjects who met the study criteria and agreed to participate were entered into the study; of these, 29 were evaluable for efficacy. Twenty subjects received a single 2 gm dose of cefotetan, and nine subjects received three 2 gm doses each of cefoxitin. Both antibiotics were administered intravenously at the time the umbilical cord was clamped. The subsequent doses of cefoxitin were given intravenously at four and eight hours after the initial dose. Clinical and bacteriologic responses were evaluated; there were no statistically significant differences between the two groups, and both antibiotics provided effective prophylaxis against infection. It appears that cefotetan is a satisfactory antibiotic choice for cesarean section prophylaxis. Further, in this small study it appears that a single dose of cefotetan is as effective as three doses of cefoxitin. This implies that cefotetan would not only decrease administration time and supplies but would decrease the cost to the patient while maintaining very acceptable infection rates. (Am J Obstet Gynecol 1986;154:951-4.)

Key words: Cefotetan, cesarean section, antibiotic prophylaxis

The past two decades have seen an increase in the incidence of cesarean sections. Although this increased cesarean section rate has been associated with diminished perinatal mortality, it has also been associated with an increase in maternal postoperative infections.[1] The reported incidence of endometritis occurring after primary cesarean section ranges from 29%[2] to 85%,[3] which is five to 15 times the rate observed in similar populations delivered vaginally. The majority of such obstetric postoperative infections are polymicrobial in origin and involve organisms indigenous to the reproductive tract. The most commonly isolated organisms are the aerobic and anaerobic streptococci, the aerobic gram-negative rods (the most common of which is *Escherichia coli*), and the anaerobic gram-negative rods (including *Bacteroides fragilis*).[4]

Although the use of prophylactic antibiotics for patients undergoing cesarean section has only been studied for 15 to 20 years, it has been unequivocally shown to be of benefit.[5] Whereas in the past antibiotics were administered preoperatively, they are now administered intraoperatively immediately following delivery and clamping of the umbilical cord. Multiple antibiotic regimens have been used, almost all with some success.[6] A number of drugs are currently approved for cesarean section prophylaxis by the Food and Drug Administration, and all currently require multiple administration.

Cefotetan is a new broad-spectrum cephalosporin antibiotic that is very resistant to gram-negative β-lactamases. It is active against many of the organisms commonly encountered in post–cesarean section infections.[7] Because of its broad spectrum of activity, its reported high concentration in uterine tissue, and its extended mean elimination half-life, cefotetan could be a potentially useful agent in the prevention of post–cesarean section infections. The decision was therefore made to compare cefotetan in a randomized prospective blinded fashion against a commonly used and approved antibiotic (cefoxitin sodium). The objectives of the study were (1) to evaluate the safety, tolerance, and prophylactic effectiveness of cefotetan in reducing the incidence of postoperative infections associated with cesarean section and (2) to obtain information on plasma levels of cefotetan after intravenous administration.

Material and methods

The study protocol was reviewed and approved by the University of Iowa Hospitals and Clinics Institutional Review Board for Experiments Involving Human Subjects. Written informed consent was obtained from all patients.

Inclusion criteria were considered for all hospitalized adult female patients scheduled to undergo primary or repeat cesarean section. These criteria included age of 15 to 50 years, a willingness to forego breast-feeding

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Table I. Indications for exclusion of randomized patients

	Cefotetan	*Cefoxitin*
Total randomized	24	12
Nonevaluable for efficacy	4	3
Infection prior to surgery	1	2
Antibiotics prior to surgery	3	1
Total evaluable for efficacy	20	9

Table II. Demographic characteristics—evaluable patients

	Cefotetan (n = 20)	*Cefoxitin (n = 9)*
Age, mean (yr)	23.5	21.1
Race		
Caucasian	18	6
Other	2	3
Duration of pregnancy		
Term	12	3
Premature	8	6
Primary cesarean section	10	2
Repeat cesarean section	10	7

for at least 48 hours after antibiotic administration, and the absence of clinical or bacteriologic evidence of infection. Asymptomatic bacteriuria was not considered an exclusion.

Exclusion criteria included (1) an oral temperature of 100.4° F (38° C) or greater within 24 hours before surgery, (2) a history of immediate hypersensitivity reaction or other adverse reaction to penicillin or to cephalosporin-type agents, (3) known or suspected significant renal function impairment as manifested by a serum creatinine level of 2.5 mg/100 ml or greater, (4) the presence of an associated serious underlying disease or rapidly fatal condition that would make it unlikely that treatment in the study or follow-up could be completed, (5) an identifiable risk of life-threatening pseudomonas disease (for example, patient with acute leukemia or granulocytopenia), (6) the presence of obvious amnionitis as manifested by an increased white blood cell count and/or uterine tenderness (this group of patients was excluded even if afebrile), and (7) subjects receiving an antibiotic agent within 2 weeks of entering the study.

After informed consent was given, patients were randomized to receive either 2 gm of cefotetan intravenously immediately after clamping of the umbilical cord, or 2 gm of cefoxitin intravenously immediately after cord clamping. Patients in the latter group also received 2 gm of cefoxitin intravenously at 4 and 8 hours post partum. Randomization was accomplished with use of an allocation schedule provided by the Biostatistics Department of Stuart Pharmaceuticals Inc. A total of 36 patients were assigned to cefotetan or cefoxitin in a 2:1 ratio with the intent of accumulating a total of 30 evaluable patients. No other antibiotics were administered subsequently unless they were considered clinically indicated by the attending physicians.

Table III. Preoperative laboratory values*—evaluable patients

	Cefotetan (n = 20)	*Cefoxitin (n = 9)*
Hemoglobin (gm/100 ml)	12.07	12.47
White blood cell count ($\times 10^3$/mm^3)	11.51	11.13
Platelets ($\times 10^5$/mm^3)	222.95	190.83
Serum glutamic-oxaloacetic transaminase	33.19	27.06
Lactate dehydrogenase	242.27	209.39
Alkaline phosphatase	205.36†	154.89†
Total bilirubin (mg/100 ml)	0.55	0.48
Blood urea nitrogen (mg/100 ml)	12.05	9.44
Creatinine (mg/100 ml)	0.75	0.70
Prothrombin time (sec)	10.90	10.56

*All values expressed as mean values.

†Indicates statistically significant difference ($p<0.05$, Wilcoxon rank sum test).

Before surgery, all patients were evaluated with clean-catch midstream urine cultures, complete blood counts, prothrombin times, and serum chemistry studies to measure hepatic and renal function. Preoperative transvaginal cervical cultures were obtained only in women with premature rupture of the membranes. Intraoperative transabdominal cultures were obtained from the endocervix and abdominal incision. Plasma specimens were obtained between 1 and 2 hours postoperatively to assay concentrations of cefotetan. All laboratory studies were repeated at 24 hours postoperatively, and all patients were evaluated daily with particular attention to wound grading and possible adverse reactions. Appropriate material for culture was obtained from any subject who subsequently developed an infection. All patients received a final evaluation at 7 to 10 days after discharge.

Criteria for early discontinuation included development of any serious medical condition that would make it unwise in the opinion of the investigators to continue, presence of a serious or alarming adverse reaction, an opinion of the investigator that it was in the best interest of the patient to withdraw, or the patient's desire to withdraw from the study.

Response criteria were evaluated both on clinical and bacteriologic grounds. Clinical success was defined as lack of signs or symptoms of infection. Clinical failure was defined as development of signs or symptoms of infection, including fever, urinary tract infection, wound infection, parametritis/pelvic cellulitis, endometritis, pelvic abscess, septic pelvic thrombophlebitis, or bacteremia. Bacteriologic success was defined as no

Table IV. Postoperative laboratory parameters*

	Cefotetan (n = 20)	*Cefoxitin (n = 9)*
Hemoglobin (gm/100 ml)	10.69	10.50
White blood cell count ($\times 10^3$/mm^3)	11.50	10.53
Platelets ($\times 10^3$/mm^3)	235.07	222.19
Serum glutamic-oxaloacetic transaminase	36.72	29.89
Lactate dehydrogenase	274.15	263.55
Alkaline phosphatase	149.07	131.70
Total bilirubin (mg/100 ml)	0.50	0.44
Blood urea nitrogen (mg/100 ml)	8.14	6.22
Creatinine (mg/100 ml)	0.75	0.77
Prothrombin time (sec)	10.82	10.83

*All values expressed as mean values.

Table V. Clinical response—all patients

	Cefotetan (n = 24)	*Cefoxitin (n = 12)*
Maximum increase in pretreatment temperature (°F)		
Mean	2.0	2.2
Median	1.6	1.9
Range	0.1-4.5	0.6-3.9
Postoperative hospitalization		
Mean	6.1	6.4
Median	6.0	6.5
Range	4.9	5.9
Wound grading		
0	21	11
1	3	1
2	0	0
3	0	0

Table VI. Bacteriologic response—evaluable patients

	Cefotetan (n = 20)		*Cefoxitin (n = 9)*	
	n	%	*n*	%
Success	16	80	8	89
Failure	2	10	0	
Unable to determine*	2	10	1	11

*Each patient had one culture report missing.

evidence of bacteriologic pathogens in all postoperative cultures. An unsuccessful bacteriologic response was defined as the presence of $>10^5$ organisms per milliliter in a urine culture, two consecutive positive blood cultures, or the presence of potentially significant pathogens on subsequent clinically indicated cultures.

Statistical evaluation for continuous measures was accomplished with use of the Wilcoxon rank sum test. For categorized data the χ^2 analysis was used. A p value of 0.05 was assigned for minimal statistical significance.

Results

The total number of patients randomized in this study was 36, of whom 29 were evaluable for efficacy. The indications for exclusion are presented in Table I.

The demographic characteristics of the evaluable patients are presented in Table II. As can be seen, the two groups were not significantly different in any of these respects, although there were relatively higher frequencies of term delivery and primary cesarean section in the cefotetan group. The preoperative laboratory values are demonstrated in Table III. These are similar in both groups except for alkaline phosphatase. The postoperative laboratory parameters (Table IV) revealed no persistent difference in alkaline phosphatase values.

Clinical response was evaluated in both groups, and a statistical difference was not found (Table V). This included increase above pretreatment temperature, postoperative hospitalization in days, and wound grading as defined by Karl et al.[8]

Bacteriologic response was also evaluated and was not significantly different in either group (Table VI). Both bacteriologic failures in the cefotetan group involved endometrial cultures revealing enterococci in patients with clinical endometritis.

Only one adverse sign or symptom was seen in the entire study population (Table VII). A patient who received cefoxitin developed pruritus within 24 hours after drug administration. This resolved spontaneously without treatment. No adverse signs or symptoms were seen in any patient receiving cefotetan.

Plasma cefotetan concentrations were obtained for 23 patients postoperatively and are shown in Table VIII, which demonstrates adequate antibacterial levels.

Comment

No serious side effects were noted in any of the patients participating in this study. Although the cefotetan group did have significantly higher preoperative serum alkaline phosphatase values, we do not believe this represents a clinically significant difference, since the other serum chemistry values reflecting hepatic function were similar in both groups. During pregnancy, alkaline phosphatase is produced by the placenta, and larger, more mature babies tend to have larger placentas. Because there were relatively more term deliveries in the cefotetan group, we were not surprised to see a relatively greater increase in alkaline phosphatase in this group. Alkaline phosphatase values were not different at the time of postoperative laboratory assessment.

The current study represents the first comparison of

Table VII. Adverse signs or symptoms

	Cefotetan (n = 24)	*Cefoxitin (n = 12)*
None	24	11
Pruritus	0	1

Table VIII. Plasma cefotetan concentration (n = 23)

	Median	*Mean*	*Range*
Time of collection (min)	96.0	94.7	8.0-178.0
Plasma concentration (μg/ml)	85.2	85.9	56.9-140.0

a single-dose prophylactic antibiotic compared directly to a currently approved three-dose regimen. No statistically significant differences were found for any of the major variables examined. This suggests that cefotetan (2 gm intravenously in a single dose) may be as satisfactory a choice for post–cesarean section infection prophylaxis as cefoxitin (2 gm intravenously in three doses), especially if it can be administered with less expense and possibility for error in administration. Further studies with larger numbers will be required to adequately address these issues.

This study also shows that cefotetan has sufficiently high serum levels for several hours in women undergoing cesarean section, which supports its potential use as a single-dose prophylactic agent.

It was the impressionn of the authors that cefotetan allowed decreased administration time, fewer supplies, and therefore a decrease in cost, while maintaining good prophylaxis against infection. Further evaluation of this would seem warranted on the basis of these findings.

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Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients

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A prospective, randomized, open trial of a single intravenous dose of a new broad-spectrum and long-acting cephalosporin was compared with the effect of three doses of cefoxitin in a group of 70 women undergoing cesarean section who were at high risk for postoperative endomyometritis and wound infection. All patients either had ruptured membranes or were in active labor, or both, without clinically detectable chorioamnionitis at the time of prophylaxis. Forty-six women received a single 2 gm dose of cefotetan and 24 received 2 gm of cefoxitin every 4 hours to complete a three-dose regimen. Outcomes of infectious febrile morbidity due to endomyometritis (15% versus 8%), wound erythema (4% versus 12%), and other parameters were similar for cefotetan and cefoxitin, respectively. Both agents were well tolerated in this high-risk population. Within the limits of this study, single-dose cefotetan chemoprophylaxis appears to be comparable to multidose cefoxitin administration in reducing morbidity in operative site infections after cesarean section. (Am J Obstet Gynecol 1986;154:955-60.)

Key words: Cefotetan, cesarean section, endomyometritis, postoperative infection

Use of perioperative systemic antibiotic chemoprophylaxis in women who are at increased risk for postoperative infection after cesarean section has proved effective and is an increasingly accepted practice.[1, 2] Further understanding of the optimum use of chemoprophylaxis regimens that provide maximum desired effect while engendering minimum iatrogenic morbidity and costs is required.[6] Major issues requiring continued study include (1) stratification of patients at risk for infection and (2) optimization of cost-effective use and ease of administration in patients in whom antibiotic prophylaxis is indicated. Studies of clinical characteristics of women at risk for postoperative infection after cesarean section identify similar findings in a variety of settings. These include labor, ruptured membranes, and low socioeconomic status in antibiotic-treated, placebo-treated, and untreated women.[3-5] Differentiation of other host factors, including qualitative and quantitative aspects of microbiologic colonization and/or infection at the time of operation, remains to be more fully elucidated.

Much interest is presently focused on practical aspects of chemoprophylaxis administration, including the benefits of single-dose administration in comparison with multidose chemoprophylaxis.[6] Recently developed antimicrobial agents possess both antimicrobial coverage (most aerobic and anaerobic lower genital tract microorganisms) and pharmacokinetic properties that allow for extended serum and tissue levels after single administration.[7] In order to evaluate a new broad-spectrum cephalosporin that possesses these attributes in preventing post–cesarean section infection, we evaluated cefotetan in a randomized, prospective trial of prevention of postoperative infection in comparison with a three-dose regimen of cefoxitin, an established agent in this setting.

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Reprints will not be available.

Material and methods

This study was performed at University Hospital, University of Colorado Health Sciences Center, in Denver, Colorado. The study protocol was reviewed and approved by the Institutional Review Board for Experiments Involving Human Subjects. Patients who were to have cesarean section in the presence of ruptured membranes and/or labor and who denied allergy to penicillin or cephalosporin-like agents were asked to sign an informed consent before entering the study. Treatment was assigned in a ratio of 2:1 for cefotetan versus cefoxitin by use of a blinded, computer-generated, randomized schedule, which was kept in the central pharmacy. The prophylactic regimens were either a single administration of a 2 gm dose of cefotetan given intravenously immediately after cord clamping or a similarly administered 2 gm dose of cefoxitin followed by two further intravenous doses at 4 and 8 hours after operation.

The criteria for exclusion were as follows: an oral temperature ≥100.4° F (38° C) within 24 hours before operation; history of an immediate hypersensi-

Table I. Preoperative profile of patients

	Cefotetan (N = 46)	*Cefoxitin (N = 24)*
A. Demographic and reproductive data		
Age	23.6 (16-37)*	23.9 (16-39)*
Ethnicity		
White Caucasian	28 (60.9%)	14 (58.3%)
Black	6 (13%)	2 (8.3%)
Hispanic	12 (26.1%)	7 (29.2%)
Oriental	0	1 (4.2%)
Insurance rating		
Private insurance	7 (15.2%)	4 (16.7%)
Medicaid	13 (28.3%)	6 (25%)
No insurance	26 (56.5%)	14 (58.3%)
Parity		
Nulliparous	23 (50%)	11 (45.8%)
Parous	23 (50%)	13 (54.2%)
Gestational age	37.3 (27-41)*	38.5 (30-41)*
35 wk	13 (28.1%)	3 (12.6%)
37 wk	33 (71.7%)	21 (87.4%)
Maternal weight (pounds)	163.8 (121-262)*	166.5 (126-272)*
Hematocrit (preoperative)	36.2 (29-43)*	35.9 (25-41)*
B. Antepartum complications		
None	14 (30.4%)	15 (62.5%)†
Pregnancy-induced hypertension	6 (12.9%)	1 (4.2%)
Multiple gestation	4 (8.6%)	1 (4.2%)
Placenta previa	3 (6.5%)	1 (4.2%)
Premature labor	5 (10.9%)	0
Premature rupture of membranes	2 (4.3%)	0
Urinary tract infection (by history)	3 (6.5%)	2 (8.3%)
Genital herpes simplex	3 (6.5%)	0
Vaginitis	2 (4.3%)	2 (8.3%)
Maternal diabetes	2 (4.3%)	0
Other	2 (4.3%)	2 (8.3%)

*Values are mean and range.
†χ^2 = $p < 0.05$.

Table II. Risk factors for postoperative infectious morbidity by group

	Cefotetan (N = 46)	*Cefoxitin (N = 24)*
Fetal membrane status		
Intact	26 (56.5%)*	4 (16.7%)
Ruptured	20 (43.5%)	20 (83.3%)
Duration of ruptured membranes (hr)	22.11 (1.5-192)† N = 20	16.2 (2.75-120)† N = 20
No. of patients with internal monitoring		
Heart rate	13 (28.2%)	9 (37.5%)
Uterine catheter	9 (19.5%)	9 (37.5%)
Anesthesia		
General	28 (60.9%)‡	8 (33.3%)
Regional	18 (39.1%)	16 (66.7%)
Duration of operation (min)	48.9 (24-80)†	53.0 (28-90)†

*χ^2 = $p < 0.01$.
†Values are mean and range.
‡χ^2 with Yates correction, p = 0.053.

Table III. Indications for cesarean section

	Cefotetan (N = 46)		*Cefoxitin (N = 24)*	
	n	%	*n*	%
Cephalopelvic disproportion/failure to progress	12	26.1	11	45.8
Placenta previa/abruptio placentae	5	10.8	0	0
Refused trial of labor/prior uterine scar; ruptured membranes	8	17.4	4	16.7
Fetal distress	6	13.0	1	4.2
Prematurity	4	8.7	0	0
Fetal lie	4	8.7	6	25
Fetal anomalies	2	4.3	1	4.2
Multiple gestation	2	4.3	1	4.2
Active herpes simplex	3	6.5	0	0

tivity reaction to penicillins or adverse reactions to cephalosporin-type agents; known or suspected renal function impairment with serum creatinine ≥2.5 mg/100 ml; associated underlying disease or rapidly fatal condition that made it unlikely that treatment with the study drug or follow-up could be completed; identifiable risk of life-threatening pseudomonas dis-

Table IV. Comparison of postoperative infectious morbidity by group and membrane status*

	Cefotetan			*Cefoxitin*		
	Without infectious morbidity	*Endomyometritis*	*Wound*	*Without infectious morbidity*	*Endomyometritis*	*Wound*
Intact membranes	22	3	1†‡	3	0	1
Ruptured membranes	13§	4	3†	16	2	2†‡

*There is no significant difference for morbidity related to membrane status.

†One patient had wound infection that required surgical treatment.

‡One patient in each antibiotic group was diagnosed as having both endomyometritis and a wound infection or wound erythema; these patients are shown in both categories.

§One patient with ruptured membranes developed a preoperative urinary tract infection.

ease; obvious amnionitis manifested by increased white blood cell count or uterine tenderness, even if the patients were afebrile; administration of any antibiotic agents within 2 weeks of starting this prophylactic regimen.

All patients had a complete blood count, differential count, and platelet count, and determination of prothrombin time, blood urea nitrogen, creatinine, electrolyte, and liver enzyme blood levels, as well as urine culture and urinalysis, with specimens obtained preoperatively from the Foley catheter. These tests were repeated approximately 24 hours later. Cervical cultures for aerobic and anaerobic bacteria were obtained preoperatively. Similar cultures were obtained from the lower uterine segment subsequent to the delivery of uterine contents and from the subcutaneous wound at the time of skin closure. Specimens were collected in Anaerobic Transport Tubes (BBL, Cockeysville, Maryland) and immediately taken to the University Hospital Microbiology Laboratory where they were inoculated with a variety of standard aerobic or anaerobic media. Aerobic microorganisms were recovered by plating specimens on 5% sheep blood agar, chocolate agar, modified Martin-Lewis agar, and MacConkey's agar with all plates incubated in 5% carbon dioxide environment at 35° C. Anaerobic microorganisms were cultured by plating on anaerobic Brucella agar plates containing 5% sheep blood and supplemented with hemin and vitamin K_1. In addition, one tube of fluid thioglycollate with supplemented hemin and vitamin K_1 was inoculated. Plates were placed in an anaerobic environment with the use of a Gas-Pak generator system (Bioquest, BBL) and incubated at 35° C. Aerobic cultures were read at 24 and 48 hours and reported semiquantitatively. Anaerobic cultures were examined at 2 days for plates and 7 days for broth. Established microbiologic techniques were used to identify both aerobic and anaerobic microorganisms.[8] Sensitivities of aerobic microorganisms were determined by Kirby-Bauer techniques.[8]

Table V. Comparison of postoperative infectious morbidity by treatment group

	Cefotetan (N = 46)		*Cefoxitin (N = 24)*	
	n	%	*n*	%
Endomyometritis	7	15.2*	2	8.3
Wound infections	2	4.4	1	4.2
Wound erythema	2	4.4	3	12.5
Urinary tract infections	1	2.2	0	
Postoperative stay (mean days)	4.5		4.5	

*One patient in cefotetan group was diagnosed as having endomyometritis and wound infection.

Standardized forms were used to collect demographic, clinical, laboratory, microbiologic, and postoperative outcome data. Patients were evaluated twice daily during hospitalization. Any patient who developed an oral temperature ≥38° C on two occasions at least 6 hours apart was carefully evaluated with standard techniques for the cause of febrile morbidity.[9, 10] Standard criteria for determining postoperative infectious morbidity were used.[9] Postoperative endomyometritis was defined as fever ≥38° C with uterine tenderness and a positive endometrial culture in the absence of any other cause of infectious morbidity. Any patient with febrile morbidity of any cause had a repeat blood count and differential count as well as a urine culture and repeat endometrial culture of a specimen taken with a double-lumen catheter (Uterine Sampling Device, Medi-Tech, Watertown, Massachusetts). Roentgenographic and other diagnostic procedures were performed if clinically indicated. The usual treatment for postoperative endomyometritis despite chemoprophylaxis was a combination of ampicillin with genta-

Table VI. Number of patients with positive cultures for high- and low-virulence organisms isolated from cervix, uterus, or incision

	Cervix	*Uterus*	*Incision*
No growth	5	17	15
Low-virulence			
Aerobes			
Gram-positive			
Lactobacillus sp.	43	23	10
Staphylococcus epidermidis	44	21	43
Diphtheroids	25	6	1
Yeasts	9	3	0
Gram-negative			
Neisseria sp.	1	0	0
Gram-variable			
Gardnerella vaginalis	28	16	7
Anaerobes			
Gram-positive			
Actinomyces meyerii	1		
High-virulence			
Aerobes			
Gram-positive			
Streptococcus viridans	9	5	3
Group B streptococcus	0	1	0
Group D streptococcus	5	1	0
Staphylococcus aureus	0	0	1
Other	0	0	2
Gram-negative			
Klebsiella spp.	1	1	0
Proteus spp.	1	0	0
Escherichia coli	10	0	0
Anaerobes			
Gram-positive			
Cocci	3	4	0
Rod	2	0	0
Gram-negative			
Bacteroides spp.	1	0	0
Bacteroides ureolyticus	1	1	0
Fusobacterium	1	1	0
Mixed	1	5	0
Rod	4	5	0
Mixed	7	4	2
Total No. of women with low-virulence isolates	64 (70)	51 (53)	53 (55)
Total No. of women with high-virulence isolates	26	13	2

micin or these drugs plus clindamycin or gentamicin and clindamycin, all given intravenously. Data were analyzed by the computer programs of the Statistical Package for the Social Sciences on an IBM XT computer. Standard statistical methods for analysis of variance, Student's t test and χ^2 for parametric data, and Mann-Whitney U testing for nonparametric data were used.

Results

Seventy patients underwent cesarean section and were evaluated. Forty-six received a single 2 gm dose of cefotetan and 24 received three 2 gm doses of cefoxitin. There were no significant differences among treatment groups for age, race, socioeconomic status (as measured by method of payment), or individual medical characteristics as noted in Table I. Various risk factors for postoperative infectious morbidity were evaluated. There were no significant differences between the treatment groups in occurrence of these factors, other than the presence of ruptured membranes, as noted in Table II. Operative indications are listed in Table III. The similarity between the treatment groups allows for comparison of outcomes. Analysis of the effect of ruptured membranes for each treatment group was carried out separately (Table IV).

Comparative results for postoperative infectious morbidity are summarized in Tables IV and V. There were no significant differences between the test regimens in the occurrence of febrile morbidity, endomyometritis, wound infection, wound erythema, urinary tract infection, or other study parameters. The presence of endomyometritis, wound infection, or wound erythema did not differ significantly with membrane status for either treatment group (Table IV). There were small numbers of infectious complications in both groups.

Preoperative cultures revealed the presence of nor-

Table VII. Predominant organisms isolated before, during and after operation from patients who developed endomyometritis (N = 9)

	Cervix	*Uterus*	*Incision*	*Endometrium*
No growth	1	0	3	1
Lactobacillus sp.	6	5	0	5
S. epidermidis	3	2	6	1
Streptococcus, nonhemolytic	3	0	0	1
Gardnerella vaginalis	6	5	2	4
Streptococcus				
S. viridans	1	2	1	2
Group D	0	0	0	4
S. pyogenes	0	0	0	1
E. coli	1	0	0	0
Mixed anaerobic flora	1	0	0	1
Anaerobic gram-positive cocci	1	0	0	0
Anaerobic gram-negative rod	0	0	0	1
Yeasts	1	0	0	1

Two additional patients had wound infections. One of these had group D streptococci isolated from the cervix but not the uterus or incision. Endometrial cultures were not obtained from patients with wound infections, unless uterine symptoms were present.

mal cervical-vaginal microorganisms at the cervix or no growth in all women (Table VI). Microorganisms (one to four isolates) were recovered from the lower uterine segment in 53 of 70 procedures (75.7%). A total of 98 isolates was recovered from the lower uterine segment from 53 patients (1.8 isolates each). One or more (one to three) microorganisms were recovered from subcutaneous tissue from 55 patients (78.3%) treated with either medication. Microorganisms isolated before, during, and after operation from patients who developed postoperative endomyometritis are listed in Table VII. All of these microorganisms were susceptible to both cefoxitin and cefotetan except group D streptococcus. All patients with postoperative morbidity due to endomyometritis responded to treatment.

No patient developed a pelvic abscess, and there were no episodes of pelvic thrombophlebitis. There were no untoward side effects from either regimen, and laboratory tests revealed no significant alterations. Postpartum clinical and chart review revealed no delayed infectious morbidity at 6 weeks other than a single case of endomyometritis in the cefotetan group and a single wound infection in the cefoxitin group.

Comment

Development of operative site infection subsequent to cesarean section depends on several factors that include the number of microorganisms within the wound, virulence of these microorganisms, presence of substrate to support bacterial growth, and the efficacy of host factors in dealing with these microorganisms.[11] Cesarean section predisposes patients to significant risks of infectious morbidity because of the "clean-contaminated" and relatively traumatic nature of the procedure. The presence of potentially pathogenic cervical-vaginal microorganisms within the lower uterine segment and amniotic fluid at the time of cesarean section in women in labor or with ruptured or even intact membranes has been demonstrated.[12, 13] In the process of surgical entry of the uterine cavity and during the subsequent closure, these microorganisms gain access to the myometrial incision as well as the abdominal wound as shown here. The relatively extensive suturing associated with most cesarean section closure techniques assures that there is devitalized tissue along with considerable foreign suture material that alters local homeostasis and impairs host defenses.

Cumulatively, these processes lead to rates of postoperative endomyometritis as high as 85% to 95% after cesarean section in high-risk patients.[5, 12, 14] Potentially lethal complications, such as septic shock, abscess formation, and pelvic thrombophlebitis, may occur in up to one of three high-risk women.[14] The antibiotic treatment results in this study are comparable to those with similar agents in placebo-controlled studies of chemoprophylaxis. This study demonstrates the comparable efficacy and safety of cefotetan when compared with cefoxitin. While costs were not detailed in this analysis, it is reasonable to suppose that a single-dose regimen would be less expensive than a three-dose regimen if these agents are comparably priced. Similarly, a single exposure to an antimicrobial agent may be less likely than three consecutive doses to cause selective pressure on endogenous microflora. The results of this small study are compatible with cefotetan's broad-spectrum and extended antimicrobial activity. We suggest that cefotetan and cefoxitin are comparable in terms of efficacy and safety, but cefotetan possesses the relative advantage of being effective after a single administration in high-risk cesarean section patients.

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Cefotetan versus cefoxitin as prophylaxis in hysterectomy

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Seventy hospitalized women were entered into an open, randomized, parallel study of antimicrobial prophylaxis for abdominal and vaginal hysterectomy with the use of either cefotetan, a new long-acting cephalosporin, or cefoxitin. Ninety-four percent of cefotetan recipients and 83% of cefoxitin recipients did not develop clinical signs or symptoms of infection. The satisfactory bacteriologic response rates were significantly different: 91% satisfactory (43 of 47 patients) for cefotetan and 74% satisfactory (17 of 23 patients) for cefoxitin. The two treatment groups demonstrated no significant differences in postoperative temperature changes, duration of hospitalization, or grading of surgical wounds. Neither drug was associated with significant side effects. (Am J Obstet Gynecol 1986;154:960-3.)

Key words: Cefotetan, hysterectomy, antibiotic prophylaxis, postoperative infection

Prophylaxis with parenteral antibiotics has been reported to reduce the incidence of infectious morbidity in gynecologic surgical procedures. Although individual risks of pelvic infection may be related to numerous variables, such as age, duration of operation, indication for operation, and use of vasoconstrictors,[1,2] the efficacy of antimicrobial prophylaxis during vaginal surgical procedures is detailed in almost all reports. The efficacy of such prophylaxis during abdominal operations is less well established.[3]

This report details a randomized prospective comparison of the safety, tolerance, and effectiveness of a single dose of cefotetan, a new, broad-spectrum, long-acting cephalosporin, and of multiple (three-dose) perioperative doses of cefoxitin.

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Material and methods

The study protocol was reviewed and approved by the University of Alabama Medical Center Institutional Review Board for Experiments Involving Human Subjects. Written informed consent was obtained from all patients.

Included in this protocol, carried out during a 14-month period, were 79 hospitalized women undergoing nonradical abdominal or vaginal hysterectomy with or without colporrhaphy. Patients were excluded from the trial on the following bases: an oral temperature of ≥100.4° F during the 24 hours preceding operation; a history of allergy or adverse reactions to pen-

Table I. Patient population

	Cefotetan	*Cefoxitin*
No. of patients	54	25
Age (yr)		
Mean	36.7	35.8
Range	22-76	23-57
Race (% Caucasian)	50	40
Vaginal hysterectomy (n and %)	38 (70)	19 (66)
Abdominal hysterectomy (n and %)	16 (30)	6 (24)

icillins or cephalosporins; renal function impairment (serum creatinine >2.5 mg/100 ml); severe hepatic, hematologic, or other systemic disorders; a history of chronic pelvic infection; a history of pelvic operation within the 2 weeks preceding hysterectomy; treatment with antibiotics within 1 week of starting prophylactic treatment. Also excluded were all patients who had urinary tract infections ($>10^5$ bacteria) on admission.

The 79 study subjects were randomized to a treatment group in a ratio of two cefotetan recipients to one cefoxitin recipient. Fifty-four patients received a 2 gm intravenous injection of cefotetan 30 to 60 minutes before operation. Twenty patients received a 2 gm intravenous injection of cefoxitin 30 to 60 minutes before the initial incision and two 2 gm doses administered intramuscularly at 6 hour intervals after operation.

Urine and uterocervical cultures were obtained before treatment. Evaluation of urine cultures included bacterial isolation, identification, and a quantitative count, as well as sensitivity testing. Uterocervical culture evaluation included bacterial isolation, identification, and sensitivity testing. Specimens were transported in anaerobic specimen containers (Vacutainer Anaerobic Specimen Collector, Becton Dickinson and Company, Rutherford, New Jersey) and were plated on selective or nonselective media within 4 hours of collection. All plates were incubated at 35° C at appropriate atmospheric conditions: chocolate agar in 5% to 10% carbon dioxide, blood agar and eosin methylene blue agar in an anaerobic environment (Gas Pak Anaerobe System, BBL, Cockeysville, Maryland). Aerobic organisms were identified by conventional microbiological methods, including the API 20E system for gram-negative bacteria (Analytab Products, Plainview, New York). Anaerobic organisms were identified by the API 20A anaerobe system (Analytab Products).

The antibiotic sensitivity testing of aerobic organisms was determined by the standard disk diffusion method with the use of a 30 mcg cefotetan disk.

During operation, plasma and uterine tissue specimens were collected and frozen for the determination of cefotetan levels. Cultures of hysterectomy abdominal and vaginal wounds were obtained at the time of closure; aerobic and anaerobic pathogens were isolated, identified, and tested for sensitivity as described.

Twenty-four hours after operation, urine and vaginal cuff cultures were repeated. Specimens for culture were also collected from other sites (incision) or when evidence of infection existed. On the third postoperative day, another culture of the vaginal cuff was obtained.

Table II. Preoperative diagnosis

	Cefotetan		*Cefoxitin*	
Diagnosis	*n*	*%*	*n*	*%*
Dysplasia	15	28	9	36
Pain, dysmenorrhea	17	31	6	24
Pelvic mass	12	22	6	24
Pelvic relaxation	4	7	–	–
Abnormal bleeding	6	11	4	16
Total	54	100	25	100

Table III. Comparison of the clinical and bacteriologic response rates

	Clinical response (%)	*Bacteriologic response (%)*
Cefotetan	94	91
Cefoxitin	83	74

Through daily clinical assessment of all patients, surgical wounds were graded for detection of infection, adverse systemic effects, and local intolerance.

Before treatment, laboratory testing for hematologic, hepatic, and renal parameters was carried out. Laboratory assessment was repeated before discharge or, if hospitalization was prolonged, performed weekly. Seven to 10 days after discharge, each patient received a final evaluation. Changes in laboratory values and temperatures were analyzed for all treated patients.

The clinical response was considered successful when no signs or symptoms of infection developed during the postoperative period. The bacteriologic response was considered satisfactory when predischarge cultures contained no evidence of resistant bacteriologic pathogens.

Results

One of three surgeons performed or assisted at each of the procedures. Preoperative hospitalization (<2 days) was minimized; preoperative hair removal, if necessary, was performed just before operation. No drains were used, and small polyglycolic acid sutures were used in 80% of patients.

Analysis indicated that the desired 2:1 randomization ratio (54 cefotetan, 23 cefoxitin) was obtained. Forty-seven of the cefotetan recipients (15 abdominal hysterectomy patients and 32 vaginal hysterectomy patients) and 23 of the cefoxitin recipients (5 abdominal hysterectomy patients and 18 vaginal hysterectomy patients) were evaluable. Nine patients (seven cefotetan, two cefoxitin) were excluded from evaluation of clinical

Table IV. Adverse signs and symptoms

*Adverse reactions**	*Cefotetan*		*Cefoxitin*	
	All effects (No. of patients)	*Treatment-related (No. of patients)*	*All effects (No. of patients)*	*Treatment-related (No. of patients)*
Clinical effects				
Gastrointestinal				
Constipation	16	0	7	0
Flatus	7	0	3	0
Nausea	4	0	1	0
Hypersensitivity				
Fever	1	0	0	0
Increased temperature	0	0	1	0
Pruritus	0	0	1	0
Itching	3	0	0	0
Rash with itching	0	0	1	0
Thigh numbness	0	0	1	0
Central nervous system effects				
Headache	4	0	0	0
Cardiorespiratory				
Lung congestion	1	0	0	0
Chest pain	1	0	0	0
Blood pressure increased	0	0	1	0
Miscellaneous clinical effects				
Back pain	1	0	0	0
Abnormal laboratory values				
Hematopoietic				
Hemoglobin decreased	1	0	0	0
Hematocrit decreased	2	0	0	0

*Some patients had more than one adverse reaction.

or bacteriologic response: six who received nonstudy antimicrobials after operation, two who were entered in an investigational pain study, and one who had positive pretreatment urine cultures. All 79 study subjects were included in the analyses of laboratory parameters, postoperative temperature, duration of hospitalization, and adverse signs and symptoms. Surgical wounds were graded only in those patients undergoing abdominal hysterectomy.

There were no statistically significant differences between the two treatment groups in the categories of race, age, diagnosis, surgical procedure, general patient condition, or serum creatinine levels (Table I). Indications for operation were not different (Table II). The cefotetan group had, on the average, a lower preoperative temperature (98.4° versus 98.7° F) and a higher preoperative alkaline phosphatase level (72.9 versus 62.72 mU/ml). Although these were statistically significant differences, both factors were well within the normal range.

In 94% of cefotetan recipients and 83% of cefoxitin recipients, no clinical signs or symptoms of infection developed (Table III). Among patients receiving cefotetan, two (4.2%) developed pelvic cellulitis and one (2.1%) developed bacteriologic evidence of urinary infection. Among cefoxitin patients, one (4.3%) required additional antibiotics for postoperative cellulitis and three (13%) had bacteriologic evidence of a postoperative urinary infection. No patients developed abdominal wound infection, pelvic abscess, septic pelvic thrombophlebitis, bacteremia, or pneumonia.

There were no statistically significant differences in postoperative temperature, duration of hospitalization, or grading of surgical wounds. Forty-three of 47 (91%) evaluable patients who received cefotetan and 17 of 23 (74%) cefoxitin recipients had satisfactory bacteriologic responses (Table III). This difference was statistically significant (χ^2 = $p < 0.05$).

A total of 39 patients (28 cefotetan 11 cefoxitin) reported adverse signs and symptoms. Of the 57 reported adverse effects (41 cefotetan, 16 cefoxitin), none were considered to be related to treatment (Table IV).

Median plasma cefotetan concentration, as measured 65 minutes after a 2 gm intravenous administration, was 149.5 μg/ml (range 1.7 to 215 μg/ml). The median uterine tissue concentration of cefotetan, as measured at a median time of 59 minutes, was 89.0 μg/gm (range 3.5 to 216 μg/gm).

Comment

Cefotetan, a semisynthetic derivative of oganomycin G produced by *Streptomyces oganonesis*, is stable to β-lactamases of gram-negative bacteria and is particularly active against *Escherichia coli, Hemophilus influenzae, Kleb-*

siella sp., *Proteus mirabilis,* and indole-positive *Proteus.* Like many other cephalosporins, cefotetan is not active against *Streptococcus faecalis* or *Pseudomonas.*[4]

In vitro testing for cefotetan activity against anaerobic bacteria that complicate gynecologic operations indicated that for 90% of strains of anaerobic gram-positive pathogens of the *Peptococcus* and *Peptostreptococcus* species, the minimum inhibitory concentration was 2 mcg/ml.[5] Moreover, 83% of *Bacteroides fragilis* isolates were inhibited by concentrations of 8 mcg/ml or less of cefotetan.[5] However, the National Committee on Clinical Laboratory Standards recommendations have set the breakpoint of cefotetan at 16 mcg/ml.

Human pharmacokinetic studies with cefotetan have demonstrated that its overall mean elimination half-life in plasma is 3.3 hours, which is substantially longer than the elimination half-life of most available cephalosporins.[6]

Early clinical trials involving more than 1300 patients have indicated that cefotetan could be used to treat gynecologic infections and many others caused by gram-positive and gram-negative aerobic and anaerobic pathogens and is well tolerated. The adverse reactions reported in these studies were not life-threatening or serious, and they cannot be considered unusual for a cephalosporin antibiotic.[1]

The evidence from our study suggests that cefotetan may have considerable value as a prophylactic antimicrobial agent used to reduce the incidence of bacterial infections after abdominal or vaginal hysterectomy. In the study, the use of cefotetan was associated with a statistically superior bacteriologic response. More importantly, a single 2 gm intravenous dose of cefotetan compared favorably to a multiple-dose regimen of cefoxitin. Thus this study suggests that use of cefotetan would be associated with large cost savings as well as improved prophylaxis against infection.

The long half-life of cefotetan contributed to the high plasma and tissue levels at approximately 1 hour after administration. This may be particularly important during abdominal hysterectomy, in which tissue contamination (i.e., entry into the vagina) occurs later. Additionally, this regimen is well tolerated by patients. No significant laboratory or clinical side effects resulted from cefotetan administration.

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CORRESPONDENCE

Another technique for resolution of shoulder dystocia

To the Editors:

I read with great interest the article on the Zavanelli maneuver (Sandberg EC. AM J OBSTET GYNECOL 1985;152:479-84.). This unorthodox method of resolving shoulder dystocia seems to be a wise course of action after all other better-known and less-traumatic measures have been taken. Any treatment that saves lives should be tried as a last resort and should be added to one's repertoire of tactics, but I was dismayed to find no mention of one of the most simple, safe, and effective techniques—squatting. This position, well known by midwives and used by those with little recourse to modern technology, has been documented to increase the pelvic capacity by about 30%.[1] This is probably due to the abductive force placed on the ischia (the body weight is partially supported by the adductors that originate from the ischial tuberosities) and facilitated by the pliability of the ligaments at term. A full squat, with feet flat (not on tiptoe) results in a wider pubic angle as the tuberosities are pulled up and apart. This may help free an impacted anterior shoulder.

This position is easily assumed by the mother with the help of a bar for her to grasp.[1] This bar simply fits into the same holes that normally hold the stirrups. It is possible for even a mother with light-to-moderate epidural anesthesia to use the squatting position with the aid of two or more people to support her legs and steady her sacrum.

Although desperate measures such as symphysiotomy or the Zavanelli maneuver may be of great value in a dire emergency, other approaches must be exhausted first. It is unwarranted to resort to such drastic procedures before more physiologic techniques, such as the squatting position, have been tried.

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REFERENCE

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Prostaglandins and endometriosis

To the Editors:

In their recent article, "Peritoneal fluid 6-keto prostaglandin $F_{1\alpha}$ levels in women with endometriosis" (AM J OBSTET GYNECOL 1985;152:901), Mudge et al. present additional evidence that a lack of significant difference exists between women with macroscopic endometriosis and women with no visible disease. Although the data is limited to the luteal phase levels of 6-keto $F_{1\alpha}$, their results are in agreement with other studies performed in the midfollicular phase.[1,2] These studies are in direct contrast to those by Drake et al.[3], who reported significantly elevated levels of 6-keto $F_{1\alpha}$ and thromboxane B_2 in patients with endometriosis as compared to a control group.

At the end of their discussion section, the authors incorrectly state that no single study has measured all of the principal prostaglandins. In November 1983, in this JOURNAL, we reported that peritoneal fluid levels during the proliferative phase of thromboxane B_2, prostaglandin E, prostaglandin F, prostaglandin FM and 6-keto F_1 were not significantly different in women with or without laparoscopically documented endometriosis, irrespective of the clinical stage of disease.[2] In a similar study Dawood et al.[4] also reported peritoneal fluid levels of thromboxane B_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and 6-keto $F_{1\alpha}$. This group found significantly elevated concentrations of 6-keto $F_{1\alpha}$ in the group of patients with endometriosis.

At present the issue of peritoneal fluid prostanoid levels in women with endometriosis remains unresolved. Differences in both study protocols and methods of prostanoid analysis make comparison of different studies difficult. It is hoped that in the near future further study will finally resolve this controversy.

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REFERENCES

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Reply

To the Editors:

Dr. Sgarlata correctly indicates that we have overlooked two relevant publications, and we apologize for this omission. We wish to further comment on Sgarlata's observation that Dawood et al. reported elevated peri-

toneal fluid concentrations of 6-keto prostaglandin $F_{1\alpha}$ in women with endometriosis.

Dawood et al. reported a statistically significant increase in the peritoneal fluid concentration of 6-keto prostaglandin $F_{1\alpha}$ in women with endometriosis. However, they also reported that the total peritoneal fluid volumes of women with endometriosis and of normal women are not different, and neither are the total amounts of 6-keto prostaglandin $F_{1\alpha}$. As discussed by Dawood et al. this statistical paradox casts doubt on the pathophysiologic significance of the apparent increase in 6-keto prostaglandin $F_{1\alpha}$ concentration.

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Cigarette smoking and endometrial cancer

To the Editors:

Tyler et al. (Endometrial cancer: how does cigarette smoking influence the risk of women under age 55 having this tumor? AM J OBSTET GYNECOL 1985; 151:899-905.) found the risk of endometrial cancer for women under 55 to be reduced approximately 20% among currently smoking women. This was not a statistically significant reduction, but in the discussion of their results the authors failed to point out that their data actually support previous reports of a negative association between cigarette smoking and cancer of the endometrium.[1]

As Hammond and Judd pointed out in the discussion, it is important to note that the Cancer and Steroid Hormone Study assembled an unusually young case series for endometrial cancer. Consequently only the "older" women in this study—those 50 to 54 years old—were in an age group with substantial representation in previous investigations. Among women of this age (more than half of the cases sampled) the Cancer and Steroid Hormone Study data show a particularly strong smoking effect among women who use noncontraceptive estrogens, in agreement with the results published by Weiss et al.[2]

It appears then that cigarette smoking is inversely related to the risk of endometrial cancer, at least in older or postmenopausal women. The mechanisms that might underlie these associations are not clear, but there is evidence that cigarette smoking acts to antagonize estrogen effects in women[1] and in this way may lower the risks of endometrial cancer. Another example of this antiestrogenic effect is the earlier menopause experienced by smokers—on average about 1 to 1.5 years earlier than nonsmokers. This alone could probably not explain a meaningful decrease in endometrial cancer risk, since a menopausal age shift of this magnitude would be associated only with a small change in risk.[1]

A major contribution that Tyler et al. have made is the demonstration that the effect of cigarette smoking may be weaker in younger age groups. Given that premenopausal women differ hormonally from postmenopausal women, it is not surprising to see such a modification by age of the effect of smoking. Another aspect of their work that deserves comment is the finding that ex-smokers seem to revert to the same risk status as never-smokers. This is also true for the relationship between smoking and age at natural menopause and suggests that the hormonal effects of smoking are reversible.[1] Obviously, further research is needed to confirm many of these associations.

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Testosterone levels and androgen-producing neoplasms

To the Editors:

The article by Friedman et al. entitled "Serum testosterone concentrations in the evaluation of androgen-producing tumors" (AM J OBSTET GYNECOL 1985; 153:44-9) purports to show that a level of >2 ng/ml of "testosterone" is not predictive of the high likelihood of an androgen-producing neoplasm. We would contend that their report makes quite a different point very well, that is, that there are pitfalls inherent in the use of inadequate assay techniques. Their mean testosterone levels in normal menstruating women are fully twofold higher than the levels of testosterone measured when a chromatographic separation is used rather than direct assay of the ether extract as they described. Thus half of what they have measured consists of a wide variety of cross-reacting materials, any of which could vary markedly because of factors entirely independent of the disease state. In 14 of their 18 subjects most measurements were only mildly in excess of 2 ng/ml. Only by repeat assay of these samples with use of a specific and accurate measurement of testosterone can a meaningful statement be made.

We agree completely with the authors' demonstration of the variability of circulating androgen levels and the suggestion for the measurement on a pool formed by three or four blood samples. We also agree that even with proper assay techniques there will be a rare patient with a testosterone level of >2 ng/ml, generally with hyperthecosis.

The point so well demonstrated by the authors' investigation is the danger inherent in following proposed guidelines based on the use of techniques that were not duplicated during the subsequent clinical care of patients. The temptation is great for clinical labo-

ratories to cut cost and maximize profit by using simpler but less accurate methods. We as clinicians responsible for the care of patients must exert our influence by choosing a quality laboratory. We recognize that in a university center that choice may not be readily available. Perhaps this sort of adverse experience will provide ammunition to overcome the strong forces of cost-containment in the best interests of optimal patient care.

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Reply

To the Editors:

We would agree with Dr. Meldrum's point that testosterone levels measured after chromatographic separation are generally lower than those measured directly following ether extraction. This point holds for most steroids including progesterone, which perhaps best exemplifies the trend over the past 15 years toward direct extraction assays and higher peripheral concentrations. It should, however, be acknowledged that most clinical laboratories use either direct extraction assays or newer kits involving direct assays with no extraction.

Our mean normal values were obtained at random times during the menstrual cycle, with normal menstrual cycles being the only criteria for entry. This in part is a bias for higher normal values than would be obtained using the more traditional control of nonhirsute, regularly ovulating women sampled during the early follicular phase. Recognizing the variation in laboratory values, we based our clinical recommendations on multiples of the upper range of normal and included the recommendation of a pooled sample as well as the importance of the clinical presentation.

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In defense of the vertical uterine incision

To the Editors:

Dr. Jovanovic (Am J Obstet Gynecol 1985;152:971.) holds Dr. Haesslein and myself responsible for the tendency to deliver the tiny newborn by classical cesarean section. Anyone reading Dr. Hibbard's discussion which followed my 1978 presentation of our paper would realize that our recommendations were considered controversial.[1] Besides, we now attempt to vaginally deliver these tiny babies in cul but still turn to abdominal delivery if any impending difficulty is perceived.[2]

I do not understand Dr. Jovanovic's discussion of the types of uterine incisions. It appears that he is recommending transverse classical (Kehrer) incision for many of these cases, since they often have little or no lower uterine segment. (If the patient has been treated with terbutaline, there may be a reasonable lower segment). However, the reason for abdominal delivery in my view is to achieve an atraumatic delivery of the fetus. In my association with more than 300 of these cases of newborn infants weighing <1000 gm, this can often be achieved only with a vertical uterine incision.[3]

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Reply

To the Editors:

Unfortunately, I have to disagree with Dr. Goodlin one more time, although this time on a different issue. He stated in his letter, "If the patient has been treated with terbutaline, there may be a reasonable lower segment." In other words, Dr. Goodlin claims that terbutaline creates or helps create a lower uterine segment. If terbutaline has any effect on a lower uterine segment, it is completely opposite from that claimed by Dr. Goodlin. The effect of terbutaline on the uterus is manifested by regression of the gap junction and decrease of oxytocin receptor concentration. Such effects on the uterus could only delay formation or growth of lower uterine segment, contrary to Dr. Goodlin's claim.

Going back to my paper, "Incisions of the pregnant uterus and delivery of low-birth weight infants," I would like to outline that I never recommended or even mentioned classical transverse (Kehrer) incision. I recommended a transverse upward concave incision over a partially developed lower uterine segment. This incision gives us unquestionably larger entrance to the uterus than the so-called "finger tear" of the lower uterine segment, and we do not have to overcome uterine spasm as in the case of vertical midline uterine incisions to achieve an atraumatic delivery of the fetus.

In addition to that, low transverse upward concave incisions made on even partially developed lower uterine segments appear to be durable, because of the fol-

lowing reasons, as stated in my paper: (1) "The proportion of smooth muscle in the myometrium decreases from the fundus to the cervix." (2) "Connective tissue of the uterus increases proportionally toward the cervix." (3) "The uterine structure is such that weaker points are in a longitudinal direction and that is why the most common type of tear in the unscarred uterus is a longitudinal one." (4) "The strong uterine structure is in the transverse direction of the lower part of the uterus so that even a dehiscent transverse scar sustains labor, and sometimes delivery, well."

I have opened the uterus transversely and concave upward over the partially formed lower uterine segment many times, as have many other obstetricians, but I have never observed and it has never been reported that this incision is less durable than the finger tear incision over completely formed and enormous lower uterine segment.

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Additional comment on combination conization

To the Editors:

It is with some amusement that I read the letters regarding the so-called combination conization (AM J OBSTET GYNECOL 1985;153:343-5).

I note that Drs. Grant, Rouse, and Minielly as well as Wright feel a bit remiss in that Dr. Wright was not even credited for this technique. It is odd that the Wright and Rubinstein letters have the same reference. This must, of course, be by accident.

In reviewing some of my own literature, I note that the so-called cylinder cone that Dr. Wright takes credit for was mentioned in some of our early articles and books in the early 1970s, but we did not call it a "cylinder cone." We called it a long narrow cone. We also described the so-called ring biopsy, which is really nothing new.

In 1971 we combined the ring biopsy and the cylinder cone. Unfortunately, we did not have the modern method of laser vaporization. We were forced to use the old scalpel and cautery.

I did not think it was worthwhile to publish this particular technique but talked about it at great length during many colposcopy courses. Also several of our Canadian Fellows became acquainted with the techniques before their return to Canada after spending some time at the Los Angeles County Southern California Medical Center in colposcopy and gynecologic oncology. We made slides of the technique in 1972 before most physicians even looked through a colposcope, much less used a laser.

Again, it is mind-boggling to note the obvious paranoia over such a simple process. There is no question that history repeats itself. I am sure others have used the "combination cone" without credit.

I am afraid that the credit for this technique will have to stay in the good old U.S.A., as suggested by Dorsey and Baggish.

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Maternal and fetal surveillance for older pregnant women

To the Editors:

We read with great interest the recent article by Kirz et al. (Advanced maternal age: the mature gravida. AM J OBSTET GYNECOL 1985;152:7-12.). We wholeheartedly agree that all women, regardless of age, deserve to be treated with the utmost respect, sensitivity and compassion. We also agree that pejorative terms such as "elderly gravida" should be eliminated from the obstetric literature. We are not quite sure that "mature gravida" is the best term to use as a substitute. Unfortunately, maturity does not always correlate with increasing age in either women or men.

We do not agree with the authors' interpretation of the data presented in the article, however. In careful detail they have demonstrated that women above the age of 35 years did indeed have a higher incidence of maternal and perinatal complications. Such complications included a higher incidence of hypertension, diabetes, cesarean delivery, operative vaginal delivery, breech presentation, macrosomia, congenital anomalies, and late decelerations in labor. Many of these differences in pregnancy outcome still were apparent when women with preexisting medical or obstetric complications were excluded from the study and control groups—a questionable exclusion since it is precisely the increased incidence of certain of these medical diseases in the "older" women that places them at increased risk for complications in the first place.

There is no question that maternal and fetal outcome in this series was very good. We think this is due to the excellent obstetric care provided by the authors, not to the fact that the study patients were not at increased risk. The study of Kirz et al. actually has reaffirmed our commitment to provide intensive maternal and fetal surveillance for patients in their mid to late thirties.

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Reply

To the Editors:

I am pleased that Schipul et al. were interested enough to read and comment on my article on pregnancy and advanced maternal age. I will address first what I think is the most important issue they raise: the question of analyzing a category of "healthy" older

gravid women. I certainly agree that as people age there is an increase in systemic diseases such as hypertension and diabetes. If a pregnant woman has these problems, then she should be considered to be at high risk. Of course, that is true whether she is 25 or 35 years old. These diseases alone put the patient in the high-risk category without consideration of age. More older women have these problems, therefore more older women are going to be at high risk. In that sense older women as a category are at higher risk than younger women. What about the women 35 years and older who do not have these medical problems? Are they at high risk because of age alone? Many of the previous studies have not tried to isolate age as a risk factor. The few that have do not find that age alone is a risk factor for significant perinatal problems.[1, 2] In my study the healthy gravid women (both parous and nulliparous) did not demonstrate an increase in the development of pregnancy complications. These include preterm labor, postdatism, preterm rupture of membranes, meconium, third-trimester bleeding, hydramnios, and problems with weight gain or urinary tract infections. In fact, there was an increase in preterm labor among the younger control group as compared to the older group. In regard to neonatal outcome the healthy nulliparous pregnant women did have more macrosomic babies, low 1-minute Apgar scores, and congenital anomalies. There was, however, no differences in 5-minute Apgar scores, perinatal deaths, or admissions to the neonatal intensive care unit.

The increase in congenital anomalies was probably due to the increase in chromosomal alterations with advancing age. With the healthy parous gravid patients there was no difference in any of the neonatal outcome parameters with regard to age.

In commenting about delivery modalities it is indeed true that the older group did have more operative deliveries. It is difficult to judge how many of these interventions were based on the concept of "high risk pregnancy" or "precious baby." It is possible that an obstetrician may have been more likely to expedite the delivery based on concern that this was a higher-risk pregnancy. Also, the increase in epidural anesthesia may have played a role in the use of vacuums and low forceps.

The nulliparous and parous "all" categories did demonstrate more problems with systemic diseases of aging. This was not surprising. Interestingly, preterm labor was more prevalent in the younger group with subsequent increase in low birth weight and neonatal admissions. As you pointed out in your letter, the "all parous gravidas" category did have more late decelerations, but there was no increase in meconium, low Apgar scores, or neonatal admissions for these same babies.

I suspect that a significant part of the good maternal and fetal outcome in this series was indeed related to the quality of the care provided in this tertiary care center. I, too, encourage obstetricians to maintain intensive maternal and fetal surveillance for their patients. Perhaps the message is that with our current standards of care for all of our patients there may be no significant increased risk in pregnancy for the women in their mid to late thirties.

As a final point, it was interesting to note that you object to my suggestion that we apply the adjective "mature" in describing these older gravid women. The word mature as defined in Webster's *Unabridged Dictionary*[3] has several possible definitions: (1) Completely developed or grown; fully ripe. (2) Highly developed or advanced in intellect, moral qualities, outlook, etc.; mature thinker. (3) Fully or thoroughly developed, perfected, detailed, etc.; a mature scheme. (4) Having reached its time limit; due and payable; a mature bond.

Clearly my intent in using the word mature is as in definition 1 not as in definition 2. I suspect the difference in definitions is where our disagreement lies. You stated, "unfortunately, maturity does not always correlate with increased age in either women or men." I, of course, cannot attest to development or advancement in intellect with age.

I do think that mature as referred to in my article as "having completed natural growth and development, having attained a final or desired state, condition of full development," is a preferable term to use rather than elderly, premenopausal, geriatric, etc. I am quite open to other suggestions if you have an alternative term in mind. Until then I continue to propose that we do not use terms such as elderly primigravida but instead consider a more gentle designation such as mature gravida.

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REFERENCES

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Another view of controversy about Rh prophylaxis

To the Editors:

I would like to comment on some recent correspondence (AM J OBSTET GYNECOL 1985;153:112-3). I refer to the letter, "Controversies in Rh prophylaxis," and the response by Dr. Bowman.

As a coauthor of an article on Rh isoimmunization,[1] I have an opinion to express which may or may not have some validity. I am disappointed to see in this "argument" a possible development that sounds like a

personal vendetta, with Dr. Hensleigh appearing to support an academic stance and Dr. Bowman appearing to support a clinical stance. In the first instance, one should always remember that a numerical or statistical significance does not always correspond with clinical relevance, and one could apply that to the arguments made by both of them. It seems that one can also see in this "argument" some similar analogies to the controversy regarding the safety of ultrasound. In one camp you have reputable authorities cautioning against the wider use of "routine" obstetric ultrasound studies on the grounds that the safety of ultrasound has not been proved and, on the other side, equally reputable authorities making statements that they perform routine obstetric ultrasound studies on the assumption that in the absence of any demonstrated harm, the "benefits outweight the undetermined risks."

Surely there is not one answer in Dr. Bowman's arguments that appears to have the flavor of a campaign. The additional information provided will be useful in decision making, but one must realize that in medical practice in the United States omnipotence is not well received and decisions involve (or should involve) more than just the authoritative voice of one obstetrician; therefore one could only commend Dr. Hensleigh for having developed a patient counseling tool, "Information for Rh-negative patients." In this way, a primary care obstetrician in conjunction with the patient/couple may be able to arrive at her/their own informed decision on this controversial subject.

One last issue to mention is that both arguments are defensible and it is important in the current medical legal climate in which obstetrics is practiced in the United States that, in the event that a defense is necessary (and the attacks come for either using or failing to use RhoGAM before birth), it is essential for clinicians to know they have potential defenders of their clinical practice when there continues to be such controversy.

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REFERENCE

1. Golbus MS, Stephens JD, Cann H, Mann J, Hensleigh P. Rh isoimmunization secondary to genetic amniocentesis. Prenat Diagn 1982;2:149.

A rapid method of culturing bloody amniotic fluid for chromosome analysis

To the Editors:

The amniotic fluids are routinely cultured for prenatal diagnosis of genetic disorders. At least 5% to 10% of the specimens contain maternal or fetal blood cells. The culturing of such samples is not only difficult but frequently results in unsuccessful growth. Also, the cultures initiated with bloody samples usually take longer time with the standard protocol. We made a few modifications that provide results within 10 to 15 days and wish to share our experiences with other cytogenetic laboratories.

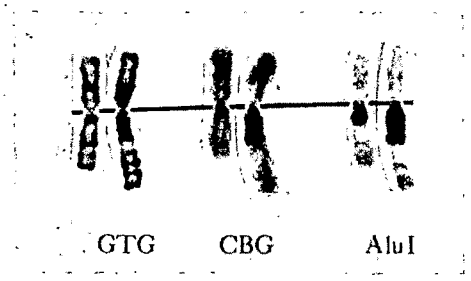


Fig. 1. Chromosome 1 from a bloody amniotic fluid with male karyotype. Results were obtained in 9 days. One of the chromosomes had an enlarged secondary constriction region (h), which was immediately confirmed by Alu I technique. Presence of this chromosome was noted in the mother.

Our culturing medium consists of the following: 100 ml of RPMI 1640 (Gibco No. 320-1875), 1.2 ml of penicillin-streptomycin (Gibco No. 600-5140), 1.2 ml of L-glutamine (Gibco No. 320-5039), and 25 ml of fetal bovine serum (Gibco No. 240-6290).

It should be emphasized that the media contain 25 ml of fetal bovine serum instead of 20 ml under normal conditions. Primary cultures should be left undisturbed in flasks for an additional 2 days, that is, up to 7 days instead of 5 days. In normal conditions the medium is changed twice a week whereas these cultures require changing at least three times a week. The cultures are usually ready to be trypsinized and the cells dispersed after the second change of medium. The reconstituted culture medium should not be more than 4 days old. The temperature of media should be 37° C before use. Although these are very minor variables, in our experience they have proved to be very important.

Another frequent problem is that certain individuals have homologues with variable lengths, whether they are polymorphic forms or chromosomal abnormalities.[1] Such incidences are more often found in chromosomes 1, 9, and 16. In these cases, the C-banding technique is being routinely used, which requires aging of the slides and consequent delay in results. Currently we have found an alternative whereby these polymorphic regions can be examined in a few hours by treating the chromosomes with restriction endonuclease Alu I (Fig. 1). We have described the elaborate method for Alu I, which can be obtained upon request.[2]

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Sterilization failures

To the Editors:

Having a considerable interest in tubal sterilization failures, I relished the opportunity to read Dr. Soderstrom's reported extensive clinical experience (Soderstrom RM. Sterilization failures and their causes. AM J OBSTET GYNECOL 1985;152:395.). I must, however, take exception to the statement made that referred to my previous publication.[1] The purpose of that study was to analyze the healing process subsequent to sterilization and consequently the cause for failures. I found no evidence that so-called "recanalization" or "endosalpingiosis" played any role. Rather, an expected normal tissue response was encountered. In the article, I also described the mechanism for the failure of the Kroener procedures, and it has nothing to do with the fimbria ovarica. The problems with the bipolar failures were recently addressed quite extensively in a series of letters to the editor in *Fertility and Sterility*.[2] The question of procedural errors was rightly pointed out. In cases that I have had the opportunity to review, some of which were impending medical-legal problems, three features stand out: (1) incomplete operative notes as far as description of equipment used, site or sites of coagulation, and the end point used for the coagulation; (2) improper handling of the excised tubal segment so that histologic reconstruction is difficult if not impossible; and (3) a failure on the part of the pathologist to recognize the coagulative effects and consequently an inappropriate final diagnosis or comment. These latter problems may lead to medical-legal action.

I wholeheartedly agree with Dr. Soderstrom's closing statement that in patients with prior sterilization failures, the tubal segments in question should be removed and studied; however, it is imperative that the segments be appropriately handled and correct histolopathologic diagnoses be made.

Richard J. Stock, M.D., M.P.H.,
Lieutenant Colonel, MC, USA

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William Beaumont Army Medical Center
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REFERENCES

1. Stock RJ. Histopathologic changes in fallopian tubes subsequent to sterilization procedures. Int J Gynecol Pathol 1983;2:13.
2. Soderstrom RM, Seiber JS, Kleppinger RK. Fertil Steril 1985;43:943.

Reply

To the Editors:

Dr. Stock is quite correct in his criticism of my reference to his article when I discussed endosalpingosis as an iatrogenic cause of sterilization failure. I meant to reference McCausland's article [McCausland A. Endosalpingosis ("endosalpingoblastosis") following laparoscopic tubal coagulation as an etiologic factor of ectopic pregnancy. AM J OBSTET GYNECOL 1982; 143:12]. My apologies to Dr. Stock and of course to Dr. McCausland.

My pathologists disagree with Dr. Stock since endosalpingosis nodosum has been present in a few of my sterilizaton failures, particularly at the proximal end of a fistula tract that formed after unipolar sterilization. Perhaps because Dr. Stock experienced electrical method failures with only two patients, a larger series might unroof a case of endosalpingosis.

I did not refer to Dr. Stock when I discussed the presence of fimbriae ovarica in all nine patients with failure of fimbriectomy sterilization. It was quite clear to me that the presence of a healthy fimbria ovarica would enhance ovum pickup and transfer to the patent tubal stump.

The three features he referred to when discussing his medicolegal experience are the same features that have been present during my medicolegal experience.

Richard M. Soderstrom, M.D.
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Books received

Biology of Women. Second Edition. Ethel Sloane. 658 pages, illustrated. Somerset, New Jersey, 1985, John Wiley & Sons, Inc. $19.95 (soft cover).

The Birthing Book. Catherine Keith and Debra Sperling. 256 pages, illustrated. New York, 1985, Penguin Books. $5.95 (soft cover).

Born Too Early. M. E. Redshaw, R. P. A. Rivers, and D. B. Rosenblatt. 265 pages, illustrated. New York, 1985, Oxford University Press. $9.95 (soft cover).

The Critically Ill Obstetric Patient. R. W. M. Baldwin and G. C. Hanson. 569 pages, illustrated. Philadelphia, 1985, J. B. Lippincott Company. $39.95.

Diagnosis and Management of Female Pelvic Infections in Primary Care Medicine. Edited by Sebastian Faro. 219 pages, illustrated. Baltimore, 1985, Williams & Wilkins. $29.50.

Early Breast Cancer: Histopathology, Diagnosis and Treatment. Edited by J. Zander and J. Baltzer. 321 pages, illustrated. New York, 1985, Springer-Verlag. $65.00.

Exercise in Pregnancy. Raul Artal and Robert A. Wiswell. 241 pages, illustrated. Baltimore, 1985, Williams & Wilkins. $36.50.

Exfoliative Cytopathology. Third Edition. Zuher M. Naib. 629 pages, illustrated. Boston, 1985, Little, Brown & Company. $55.00.

Gynecologic Urology and Urodynamics: Theory and Practice. Edited by Donald R. Ostergard. 626 pages, illustrated. Baltimore, 1985, Williams & Wilkins. $65.50.

Herpes, AIDS and Other Sexually Transmitted Diseases. Derek Llewellyn-Jones. Boston, 1985, Faber and Faber. $14.95 cloth; $6.95 soft cover.

Labour Ward Manual. D. T. Y. Liu, D. V. I. Fairweather. 124 pages, illustrated. Stoneham, Massachusetts, 1985, Butterworth Publishers. $24.95 (soft cover).

Lecture Notes on Midwifery. For Student Midwives and Medical Students. T. F. Redman. 384 pages, illustrated. Bristol, England, 1985, John Wright & Sons. $15.00.

A Manual of Clinical Obstetrics. Nancy Whitley. 770 pages, illustrated. Philadelphia, 1985, J. B. Lippincott Company. $39.50.

Oxytocin. Clinical and Laboratory Studies. J. A. Amico and A. G. Robinson. 448 pages, illustrated. Amsterdam, 1985, Elsevier Science Publishers. $111.00.

Pediatric and Adolescent Endocrinology. Vol. 14: Pediatric Thyroidology. F. Delange, D. A. Fisher, and P. Malvaux. 412 pages, illustrated. Basel, 1985, S. Karger. $117.25.

Practice Enhancement. The Physician's Guide to Success in Private Practice. Greg N. Korneluk. 284 pages. New York, 1985, Macmillan Publishing Company. No price listed.

Te Linde's Operative Gynecology. Sixth Edition. Richard F. Mattingly and John D. Thompson. 938 pages, illustrated. Philadelphia, 1985, J. B. Lippincott Company. $89.00.

Voluntary Sterilization: An International Fact Book. John A. Ross, Sawon Hong, and Douglas H. Huber. 118 pages, illustrated. New York, 1985, Association for Voluntary Sterilization, Inc. Free.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Tenth Annual Meeting, "Abortion Providers on the Front Line: Confronting the Challenges, Continuing the Commitment," June 8-11, 1986, Hyatt Regency at McGee Street, Kansas City, Missouri. For more information, please contact National Abortion Federation: (202) 546-9060.

Second Annual Long Island Assembly of OB/GYN—Crisis of the 80's: Medicolegal Issues in OB/GYN, May 29 and 30, 1986, Garden City Hotel, Garden City, New York. For information contact: Ann J. Boehme, Associate Director for Continuing Education, Long Island Jewish Medical Center, New Hyde Park, NY 11042. Tel.: (718) 470-8650.

Basic and Advanced Laser Surgery in Gynecology, July 31, August 1, and August 2, 1986, Sheraton Beach Inn, Virginia Beach, Virginia. For more information, please contact: Michael Baggish, M.D., Chief, Department of Ob/Gyn, Crouse Irving Memorial Hospital, 736 Irving Ave., Syracuse, NY 13210. Tel.: (315) 470-7903.

First Annual Postgraduate Course in Obstetrics and Gynecology, May 30-31, 1986, The Allentown Hilton and Conference Center, Allentown, Pennsylvania. For further information, contact: Sze-ya Yeh, M.D., Department of Obstetrics and Gynecology, The Allentown Hospital, 17th and Chew Streets, Allentown, PA 18102. Tel.: (215) 778-2412.

Gynecologic Surgical Techniques, June 12-14, 1986, Chicago, Illinois. For additional information, contact the Registrar's office. Toll-free number: Illinois 1 (800) 621-4649, outside Illinois 1 (800) 621-4651.

Cold Spring Harbor Laboratory Conference, 5th Annual Workshop on Papilloma viruses, September 3-7, 1986. Requests for registration and/or abstract materials should be forwarded to: Meetings Coordinator, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, NY 11724. Tel.: (516) 367-8346.

American Association of Clinical Anatomists presents a Course on the Applied Anatomy of the Pelvis, June 21-22, 1986, Mayo Clinic, Rochester, Minnesota. For further information, please contact: R. Ger, M.D., Winthrop University Hospital, Mineola, NY 11051. Tel.: (516) 663-2262.

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Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1986 issues from the Publisher, at a cost of $51.00 ($74.00 international) for Vol. 154 (January-June) and Vol. 155 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C. V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146, USA; phone (800) 325-4177, ext. 351.

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This fourth volume in the series, Animal Models in Fetal Medicine considers problems that are of fundamental importance in obstetrics and pediatrics. The book details and critically assesses investigation of regulation of fetal growth. It deals with the function of the fetal kidney and liver, and the measurement of blood and other body fluid volumes. Other contributions deal with placental transfer, substrate metabolism and fetal surgery in conditions of growth retardation. 216 pages. 1985. $45.

PARTURITION

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Parturition involves several physiological and biochemical systems. This volume considers the peptides of the hypothalamus and pituitary that are responsible for regulation of the fetal adrenal. Human, nonhuman primate, sheep and small animal experiments are carefully analyzed. In vitro, electron microscopy and computerized whole animal techniques are described and evaluated. The latest methods of studying fetal metabolism with nuclear magnetic resonance spectroscopy are described in detail. Together, the chapters describe how ideas on maternal and fetal function during normal and abnormal labor have been formulated. 320 pages. 1986. $50.

FETAL PHYSIOLOGY AND MEDICINE

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43 Contributors.

The second edition of this outstanding text covers all the important areas of fetal development. Contributors include Drs. J.C. Hobbins, J.D. Wilson, R.D.G. Milner, A. Jobe, P.D. Gluckman, G.C. Liggins, F.C. Battaglia and many other international leaders in perinatology. This book is essential reading for the obstetrician or neonatologist who wishes to keep up-to-date.

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This volume contains contributions from clinicians such as Drs Edwards, Steptoe and Jones who have pioneered many of the procedures described. In addition, the basic sciences underlying the practice of in vitro fertilization are discussed in detail. The experiences in different countries throughout the world and the legal aspects are also considered in depth. Various chapters cover egg collection, embryo culture methods, semen evaluation and pregnancy outcome. 346 pages. 1985. $55.

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G.V.P. Chamberlain, Ed. 23 Contributors.

The medico-legal aspects of obstetrics and gynecology are a common cause for interest and concern for the practitioner and the legal profession. Rapid and complex changes have taken place in the field of malpractice in recent years. In this volume, the major areas of obstetrics and gynecology, such as anesthesia, abortion, contraception, sterilization, fetal hypoxia, operative deliveries and home delivery are discussed. This volume is essential reading for all practitioners in the field. The time and money invested in reading this book could well avoid malpractice problems. 350 pages. 1985. $55.

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EARLY PREGNANCY FACTORS

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PERINATAL ENDOCRINOLOGY

E. Albrecht & G. Pepe, Eds. 17 Contributors.

A comprehensive review of fetal, neonatal and placental endocrinology. The authors include P.D. Gluckman, J. Rose, D.A. Fisher, C. Rosenfeld, C. Cheung, M. Seron-Ferre, J.D. Winter, E. Simpson, A. Klopper, E. Albrecht, G. Pepe, J.R.G. Challis, P.W. Nathanielsz, M. Soloff, and others who have made fundamental contributions to the rapidly expanding body of knowledge in this area. 315 pages. 1985. $40.

FETAL NEURO-ENDOCRINOLOGY

F. Ellendorff, P.D. Gluckman & N. Parvizi, Eds. 33 Contributors.

This was the first international meeting devoted to this developing field of perinatal research and this volume is a comprehensive compilation of reviews and papers by key workers studying the development of the neuroendocrine system. Topics covered include: neurogenesis in the mammalian brain, the role of the neuropeptides and neurohormones in neural development, the ontogenesis of neurotransmitter systems, fetal hypothalamic transplantation, fetal hypothalamic cultures, and many other important areas in relation to the significant neuroendocrine axes. 285 pages. 1985. $35.

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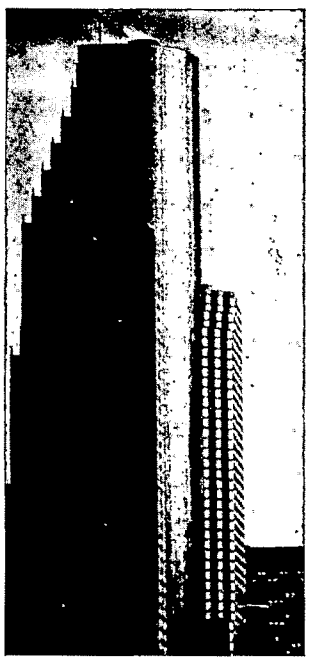
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Professor and Chairman
Department of Obstetrics, Gynecology and Reproductive Sciences
University of Texas Medical School
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James H. Lee, Jr., MD
Professor and Chairman
Dept. of Obstetrics & Gynecology
Jefferson Medical College
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Mount Sinai Medical Center
950 North 12th Street
Milwaukee, Wisconsin 53233

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Thomas G. Day, Jr., M.D.
Associate Professor
Department of Obstetrics & Gynecology
University of Louisville School of Medicine
Ambulatory Care Building
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Associate Professor/Professor Maternal Fetal Medicine

The Department of Obstetrics & Gynecology of the University of Texas Health Science Center at Dallas is recruiting for a full-time faculty position for the Division of Maternal Fetal Medicine at the Associate Professor/Professor Level. Requisites include that the applicant be board certified in Maternal-Fetal Medicine, Texas licensure, and have suitable academic credentials that include proven performance in laboratory and clinical research. The position includes a heavy clinical schedule with supervision of fellows, residents, and medical students. Interested applicants should send their curriculum vitae to:

F. Gary Cunningham, M.D.
Department of Obstetrics & Gynecology
University of Texas Health Science Center
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FULL TIME FACULTY POSITION IN THE DIVISION OF GYNECOLOGIC ONCOLOGY

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James H. Lee, Jr., MD
Professor and Chairman
Dept. of Obstetrics & Gynecology
Jefferson Medical College
Room 310 College Building
1025 Walnut St.
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The Division of Maternal-Fetal Medicine of the Department of Obstetrics and Gynecology at the University of Florida College of Medicine in Gainesville, Florida has an opening for a full-time faculty position at the Assistant to Professor rank.

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Department of Obstetrics and Gynecology
J-294, JHMHSC
University of Florida College of Medicine
Gainesville, Florida 32610

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INDEX TO ADVERTISERS

Abbott Pharmaceuticals, Inc.
Ogen 49A,50A,51A

Accurate Surgical & Scientific Instruments Corporation
Surgicraft Copeland Fetal Scalp Electrode 18A

Alexian Brothers Hospital
Opportunity Available 54A

American Society of Lipo-Suction Surgery
Workshop 52A

Astoria Clinic
Opportunity Available 54A

Berlex Laboratories, Inc.
Tri-Levlen 22A,23A,24A

Carle Clinic
Opportunity Available 54A

FHP Professional Staffing
Opportunity Available 54A

Group Health Plan
Opportunity Available 55A

Gyn Cme Fund
Seminar 35A

Harlem Hospital Center
Opportunity Available 55A

Kaiser Permanente
Opportunity Available 56A

Littleton Medical Center, P.C.
Opportunity Available 56A

Maine Medical Center
Opportunity Available 55A,56A

Marshfield Clinic
Opportunity Available 55A

Medical Equipment Designs, Inc.
HemoCue Hb Test System 14A,15A

Mead Johnson Laboratories
Estrace 30A,31A,32A

Merck Sharp & Dohme
Mefoxin 36A,37A,38A

Miles Pharmaceuticals
Mycelex-G 500 mg 8A,9A,10A

Mount Sinai Medical Center
Opportunity Available 59A

New England Memorial Hospital
Opportunity Available 61A

NME Physician Recruitment
Opportunity Available 57A

Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 .. Third Cover,Fourth Cover

Patuxent Medical Group, P.A.
Opportunity Available 57A

Pennsylvania Hospital
Opportunity Available 58A

Perinatology Press
Books 53A

Permanente Medical Group Inc.
Opportunity Available 57A

Purdue Frederick Company, The
Betadine 6A

St. John Hospital
Opportunity Available 57A

St. Joseph's Hospital—Milwaukee
Opportunity Available 58A

San Joaquin General Hospital
Opportunity Available 58A

Syntex Laboratories
Femstat 45A,46A,47A,48A
Tri-Norinyl ..... Second Cover,1A,2A

Texas Tech University
Amarillo-Opportunity Available 56A
Amarillo-Opportunity Available 58A
Lubbock-Opportunity Available 59A
Odessa-Opportunity Available 60A
Odessa-Opportunity Available 61A

Thomas Jefferson University
Opportunity Available 59A,60A

University of Florida, College of Medicine
Opportunity Available 61A

University of Louisville School of Medicine
Opportunity Available 60A

University of Texas Health Science Center at Dallas
Opportunity Available 60A

University of Texas Health Science Center at Houston
Opportunity Available 59A

Upjohn Company, The
Cleocin Phosphate 3A,4A
Provera 33A,34A

VLI Corp.
Today Sponge 12A

Wells Johnson Company
Aspirator II 20A

Wyeth Laboratories
Triphasil 39A,40A,41A,42A,43A,44A

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).**

DRUG INTERACTIONS: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

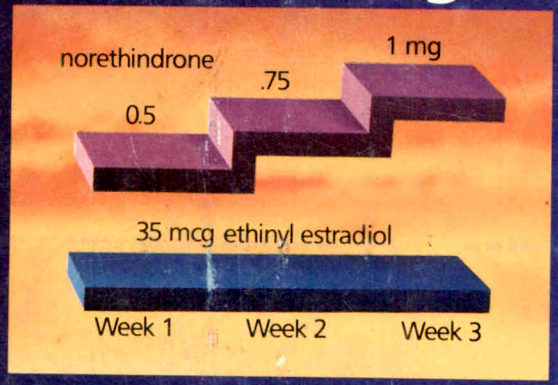
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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a summary of which appears on the preceding page.

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for both of you.



- 29% lower progestin dose than 1/35.[1]
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- Supplied in the patient-preferred Wallette™ pill dispenser.[4]

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Wallette™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.

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TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)
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ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.
MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to non-smoking users, and a 10-12-fold increased risk compared to nonsmoking non-users. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences,* or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned.
Precautions *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings.
Adverse Reactions An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

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A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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Contents

May
1986

CLINICAL SECTION

Clinical Opinion

An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis 973
Howard Minkoff, M.D., and Philip Mead, M.D.
Brooklyn, New York, and Burlington, Vermont

Perinatal events and cerebral palsy 978
Paul A. Hensleigh, M.D., Ph.D., Theodore Fainstat, M.D., Ph.D., and Roger Spencer, M.D.
San Jose, California

(Contents continued on page 7A)

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Contents continued from page 5A

Clinical Articles

Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear 982
Robert D. Burk, M.D., Anna S. Kadish, M.D., Servio Calderin, M.D., and Seymour L. Romney, M.D.
Bronx, New York

Toxic shock syndrome, contraceptive methods, and vaginitis 989
Stephan F. Lanes, Ph.D., Charles Poole, M.P.H., Nancy A. Dreyer, Ph.D., and Lee L. Lanza, M.P.H.
Chestnut Hill, Massachusetts

Necessity of endocervical curettage in colposcopy 992
Karan R. Moseley, M.D., Tung V. Dinh, M.D., Edward V. Hannigan, M.D., E. A. Dillard, Jr., M.D., and Roger B. Yandell, M.D.
Galveston, Texas

Physiologic changes in pregnant women and their fetuses during jet air travel 996
Renate Huch, M.D., Hans Baumann, M.D., Falk Fallenstein, M.Sc., Karl T. M. Schneider, M.D., Fridolin Holdener, M.D., and Albert Huch, M.D.
Zurich, Switzerland

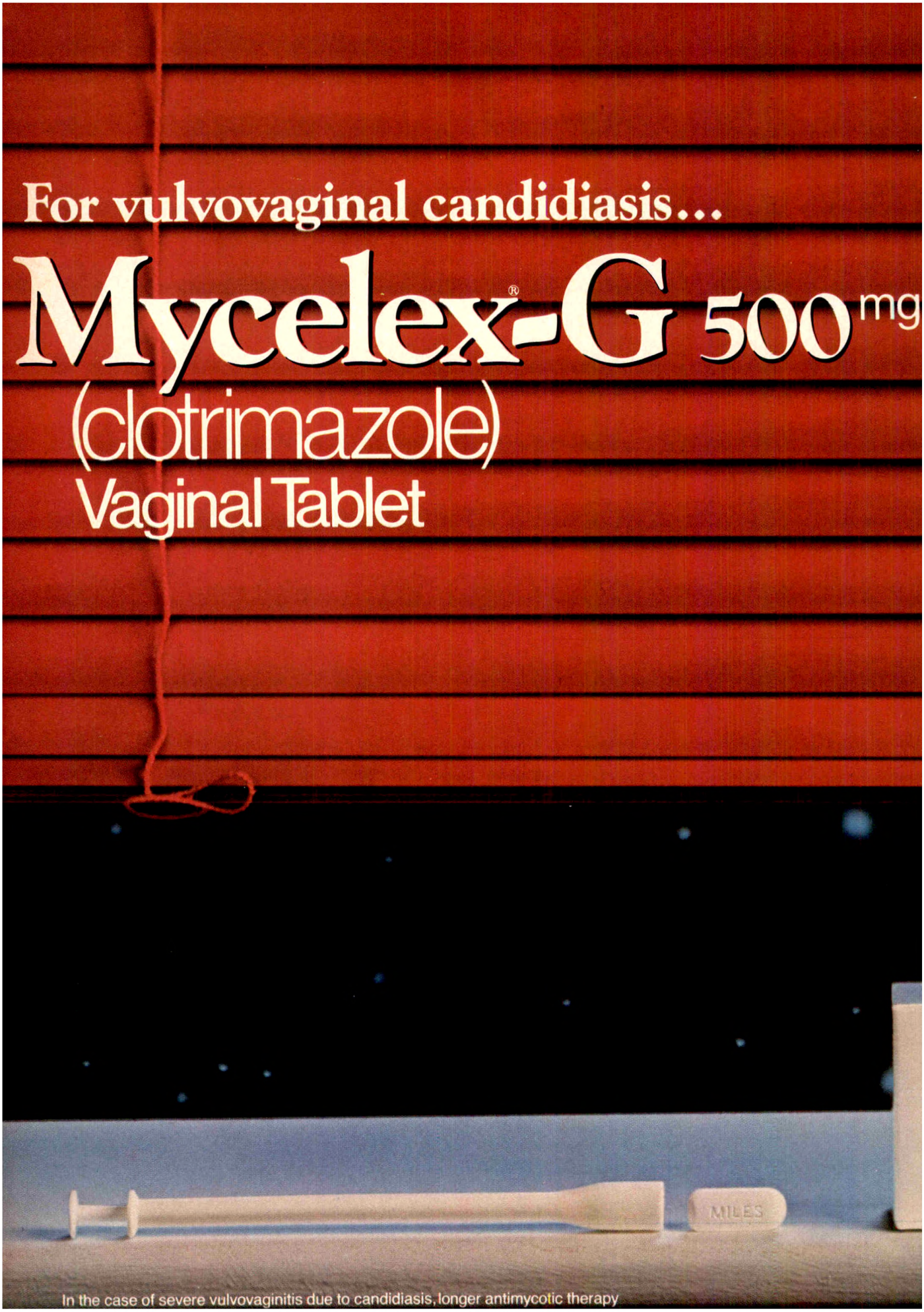
Moderate recreational running is associated with lowered salivary progesterone profiles in women 1000
Peter T. Ellison, Ph.D., and Catherine Lager, B.A.
Cambridge, Massachusetts

Are preoperative antibiotics helpful in abdominal hysterectomy? 1004
Cecilia C. Senior, M.B., B.S., B.Sc.(hons.), Dip. Ed., and Stephen J. Steigrad, M.B., B.S.
Sydney, Australia

Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods 1009
Morris Notelovitz, M.D., Ph.D., Carol Fields, M.P.E., Kim Caramelli, M.P.E., Molly Dougherty, R.N., Ph.D., and Anna L. Schwartz, B.S.
Gainesville, Florida

Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant 1014
Enrique M. Ostrea, Jr., M.D., James E. Balun, B.S., Ruth Winkler, R.N.C., and Thomas Porter, B.S.
Detroit, Michigan

(Contents continued on page 11A)


For vulvovaginal candidiasis...
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(clotrimazole)
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INDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis, *Trichomonas* and *Gardnerella (Haemophilus) vaginalis*, should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

WARNINGS: None.

PRECAUTIONS: If there is a lack of response to Mycelex®-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

CARCINOGENESIS: No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex®-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

USAGE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

ADVERSE REACTIONS: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex®-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSAGE: No data available.

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex®-G 500 mg Vaginal Tablets has not been reported.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

HOW SUPPLIED: Mycelex®-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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Contents continued from page 7A

Postpartum moods in men and women 1018
David M. Quadagno, Ph.D., Laurie Ann Dixon, M.A., Nancy W. Denney, Ph.D., and Henry W. Buck, M.D.
Lawrence, Kansas, and Madison, Wisconsin

Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios 1023
David W. Hobson, M.D., Thomas Spillman, Ph.D., and David B. Cotton, M.D.
Houston, Texas

Serum fructosamine: A screening test for diabetes in pregnancy 1027
Alistair B. Roberts, M.D., and John R. Baker, M.B.
Auckland, New Zealand

Should the fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? 1031
Donald R. Coustan, M.D., John A. Widness, M.D., Marshall W. Carpenter, M.D., Lee Rotondo, R.N., Doreen Chin Pratt, R.D., and William Oh, M.D.
Providence, Rhode Island

Decreased fetal cord prolactin concentration in diabetic pregnancies 1035
Daniel H. Saltzman, M.D., Robert L. Barbieri, M.D., and Fredric D. Frigoletto, Jr., M.D.
Boston, Massachusetts

Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers 1039
Robert L. Barbieri, M.D., Daniel H. Saltzman, M.D., John S. Torday, Ph.D., Rebecca W. Randall, B.Sc., Fredric D. Frigoletto, M.D., and Kenneth J. Ryan, M.D.
Boston, Massachusetts

Hypertensive diseases of pregnancy and parity 1044
Norbert Gleicher, M.D., Leo R. Boler, Jr., M.D., Marija Norusis, Ph.D., and Alfonso Del Granado, M.D.
Chicago, Illinois

Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic 1050
John Lazarchick, M.D., Thomas M. Stubbs, M.D., Lydia Romein, B.A., J. Peter Van Dorsten, M.D., and C. Boyd Loadholt, Ph.D.
Charleston, South Carolina

(Contents continued on page 14A)

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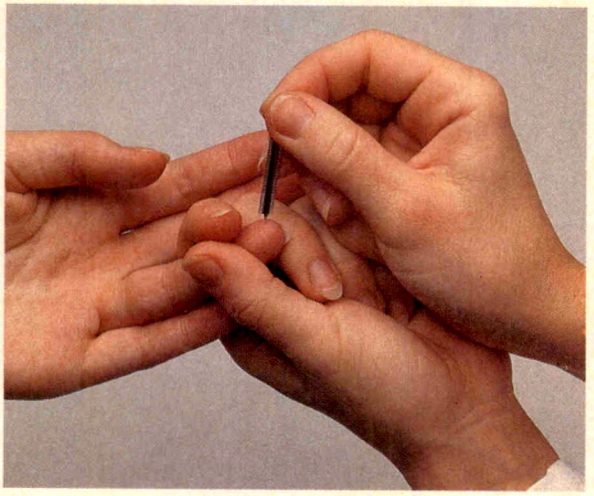
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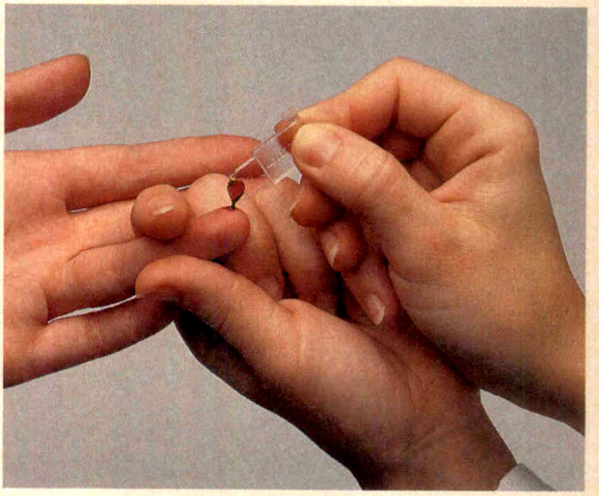
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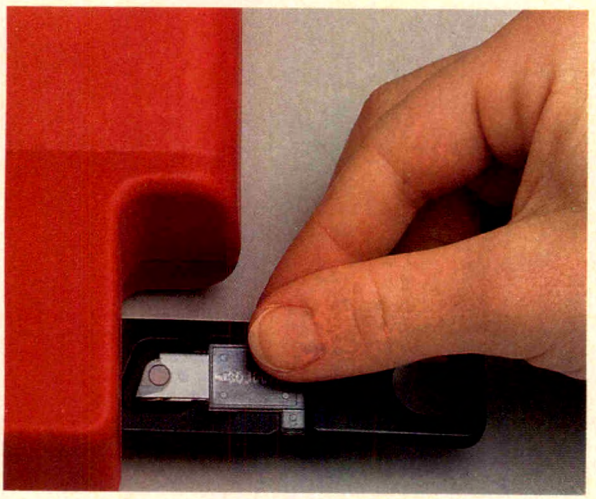
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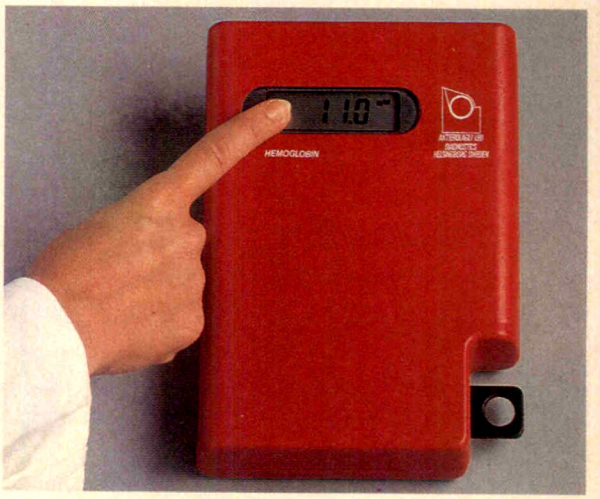
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Contents continued from page 11A

Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion 1053
David B. Cotton, M.D., Stephen Longmire, M.D., Monica M. Jones, M.D., Karen F. Dorman, R.N., Joy Tessem, M.D., and Thomas H. Joyce III, M.D.
Houston, Texas

A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes 1059
Fernando Arias, M.D., Ph.D., Alfred B. Knight, M.D., and Paul B. Tomich, M.D.
St. Louis, Missouri

Reported sexual assault in Nashville–Davidson County, Tennessee, 1980 to 1982 1064
Peter S. Cartwright, M.D., and the Sexual Assault Study Group
Nashville, Tennessee

Ultrasound evaluation of polyhydramnios and twin pregnancy 1069
Beverly Hashimoto, M.D., Peter W. Callen, M.D., Roy A. Filly, M.D., and Russell K. Laros, M.D.
San Francisco, California

The role of lymphangiography in vulvar carcinoma 1073
Sheldon A. Weiner, M.D., Joseph K. T. Lee, M.D., Ming-Shian Kao, M.D., and Thomas E. Moon, Ph.D.
St. Louis, Missouri, and Tucson, Arizona

Human fallopian tube contains placental protein 14 1076
Mervi Julkunen, M.D., Torsten Wahlström, M.D., and Markku Seppälä, M.D.
Helsinki, Finland

The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells 1080
Jacob Moise, M.D., John Ilekis, B.S., Antonio Scommegna; M.D., and Robert Benveniste, Ph.D.
Chicago, Illinois

The length of the human umbilical cord in vertex and breech presentations 1086
Torgrim Soernes, M.D., and Trygve Bakke, M.D.
Nordbyhagen, Norway

The tumor marker CA 125 is a common constituent of normal cervical mucus 1088
Henk W. A. de Bruijn, Ph.D., Ton van Beeck Calkoen-Carpay, Siemen Jager, Ph.D., Jitze M. Duk, M.D., Jan G. Aalders, M.D., and Gert Jan Fleuren, M.D.
Groningen and Leiden, The Netherlands

Contents

Human chorionic gonadotropin in early normal and pathologic pregnancy 1091
Johan Arnt Steier, M.D., Roar Sandvei, M.D., and Ole L. Myking, M.D.
Bergen, Norway

Factors related to the increasing cesarean section rates for cephalopelvic disproportion 1095
Eliot L. Silbar, M.D.
Highland Park, Illinois

Effects of maternal transport on admission patterns at a tertiary care center 1098
Richard M. Cowett, M.D., Donald R. Coustan, M.D., and William Oh, M.D.
Providence, Rhode Island

Septic shock with renal failure after chorionic villus sampling 1100
Alicia I. Barela, M.D., Gary E. Kleinman, M.D., Ira M. Golditch, M.D.,
David J. Menke, M.D., W. Allen Hogge, M.D., and Mitchell S. Golbus, M.D.
San Francisco, California

Relationship of baseline fetal heart rate to gestational age and fetal sex 1102
Maurice L. Druzin, M.D., J. Milton Hutson, M.D., and Terri G. Edersheim, M.D.
New York, New York

Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon 1104
Mortimer R. Camiel, M.D.
Brooklyn, New York

Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization 1105
John W. Seeds, M.D., and Watson A. Bowes, Jr., M.D.
Chapel Hill, North Carolina

Factors influencing nonstress test results in multiple gestations 1107
Peter Patkos, M.D., Marc Boucher, M.D., Paula M. Broussard, R.N.,
Jeffrey P. Phelan, M.D., and Lawrence D. Platt, M.D.
Los Angeles, California

Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death 1109
R. L. A. Erskine, M.B., J. W. K. Ritchie, M.D., A. Zaltz, B.Sc., M.D.,
and T. Tice, R.N.
Toronto, Ontario, Canada

(Contents continued on page 17A)

Natalins® Rx

Multivitamin and Multimineral Supplement with 1 mg Folic Acid

The Only Prenatal Vitamin with No Artificial Color or Flavor from Synthetic Sources

The Recipe for Prenatal Good Health

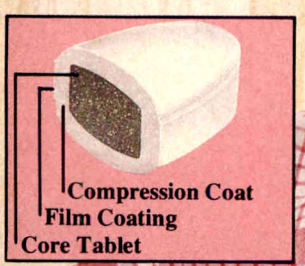
Good things are made from the finest ingredients. And the ingredients for maintaining good prenatal health make NATALINS Rx a standout . . . inside and out.

NATALINS Rx is the first and only prescription vitamin and mineral supplement with "natural" color and flavor. It contains all the mandatory and optional vitamins and minerals, plus copper and zinc, both important in the metabolic process.

Unique NATALINS Rx Construction Aids Compliance and Maintenance of Good Health

An elliptical tablet shape and a cellulosic film coating that becomes slippery when wet allows for easier, more comfortable swallowing—aiding patient compliance. Beneath the special coating, a central "core tablet" stores iron for delayed release, reducing the risk of stomach upset.

NATALINS Rx . . . the "Natural" Choice for Prenatal Good Health.



NATALINS® Rx

Ingredients	Natalins Rx	
VITAMINS	**Units**	**% RDA**
Vitamin A, IU	8000	100
Vitamin D, IU	400	100
Vitamin E, IU	30	100
Vitamin C, mg	90	150
Folic Acid, mg	1	125
Thiamine (B_1), mg	2.55	150
Riboflavin (B_2), mg	3	150
Niacin, mg	20	100
Vitamin B_6, mg	10	400
Vitamin B_{12}, mcg	8	100
Biotin, mg	0.05	16
Pantothenic Acid, mg	15	150
MINERALS		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron,mg	60	333
Magnesium, mg	100	22
Copper, mg	2	100
Zinc, mg	15	100
Artificial color	none	
Artificial flavor	none	

Natalins Rx is a multivitamin, multimineral supplement for pregnant or lactating women. Daily dose: 1 tablet or as prescribed.
Contraindications: Natalins Rx should not be prescribed in patients with hemochromatosis or patients with Wilson's disease.
Caution: Pernicious anemia should be excluded before using this product. In patients with kidney stones, the calcium content of Natalins Rx tablets should be considered before prescribing.
Supplied: Bottles of 100 and 1,000.

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N-1-85

Contents continued from page 15A

The in vivo effect of amniotic fluid on endocervical microflora 1111
Robert M. Patterson, M.D., Jorge D. Blanco, M.D., Ronald S. Gibbs, M.D., and P. J. St. Clair
San Antonio, Texas

Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section 1113
Vijaya Ganesh, M.D., Joseph J. Apuzzio, M.D., Benjamin Dispenziere, M.D., Kiritkumar Patel, M.D., Blair Bergen, M.D., and Donald B. Louria, M.D.
Newark, New Jersey

Hydrops fetalis and chromosomal trisomies 1114
Bonnie G. Landrum, M.D., Dana E. Johnson, M.D., Ph.D., Bruce Ferrara, M.D., Stephen J. Boros, M.D., and Theodore R. Thompson, M.D.
Minneapolis and St. Paul, Minnesota

Excretion of varicella–herpes zoster virus in breast milk 1116
Irene B. Frederick, M.D., Roberta J. White, Ph.D., and Suzanne W. Braddock, M.D.
Omaha, Nebraska

Folate deficiency presenting as pancytopenia in pregnancy 1117
Francis X. Solano, Jr., M.D., and R. Bruce Councell, M.D.
Pittsburgh, Pennsylvania

Gaucher's disease in pregnancy associated with portal hypertension 1119
Moshe Mazor, M.D., Arnon Witznitzer, M.D., Abraham Pinku, M.D., Miriam Katz, M.D., and Joseph R. Leiberman, M.D.
Beer-Sheva, Israel

Current Development

Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography 1121
Joshua A. Copel, M.D., Gianluigi Pilu, M.D., and Charles S. Kleinman, M.D.
New Haven, Connecticut

BASIC SCIENCE SECTION

Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus 1133
Mary M. L. Lee, Ph.D., and Ming-neng Yeh, M.D.
New York, New York

(Contents continued on page 20A)

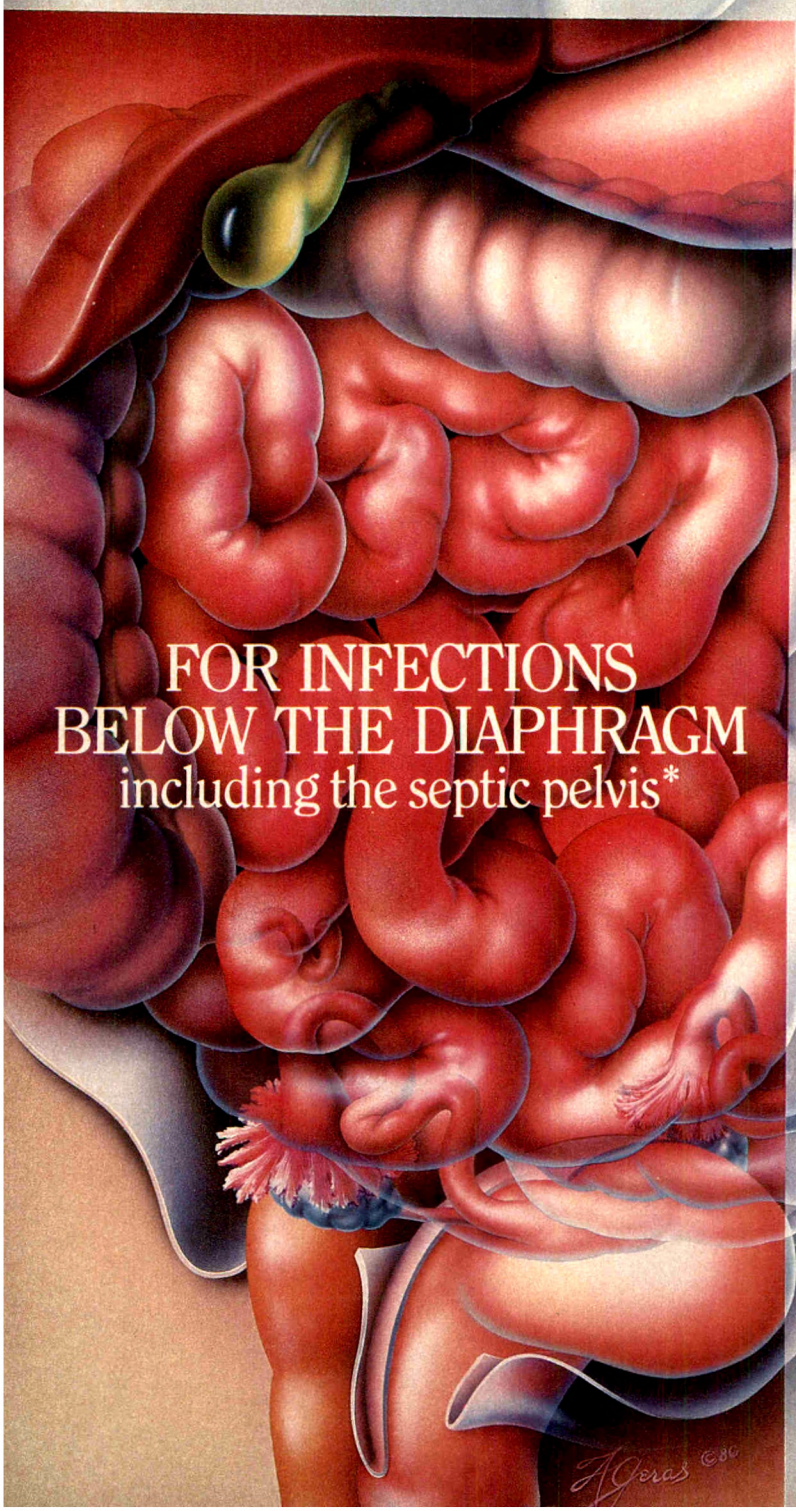
Outstanding mixed aerobic/anaerobic coverage

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brand of sterile

ceftizoxime sodium

Injection: 1 and 2 gram vials



- **B.I.D. Dosing† for All but Life-Threatening Infections**
- **Clinical Cure Unsurpassed By Clindamycin/Tobramycin[1]**

  A recent, prospective, randomized, comparative trial demonstrated that 'Cefizox' was equal to clindamycin/tobramycin in the treatment of intra-abdominal infections.
- **Better Gram-negative and *Bacteroides* Coverage Than Mefoxin® (cefoxitin)‡**

Aerobic Organisms[2] (number of strains)	CEFIZOX® (ceftizoxime) MIC_{90}	MEFOXIN® (cefoxitin) MIC_{90}
Gram-Negative	mcg./ml.	mcg./ml.
Escherichia coli (40)	0.04	6.3
Klebsiella pneumoniae (32)	0.05	25
Enterobacter aerogenes (16)	0.12	>400
Enterobacter cloacae (16)	0.8	400
Proteus sp. (indole positive) (26)	6.3	12.5
Citrobacter sp. (32)	0.2	400
Gram-Positive	mcg./ml.	mcg./ml.
Staphylococcus aureus (16)	3.1	3.1

Bacteroides species[3] (number of strains)	CEFIZOX® (ceftizoxime) MIC_{90}	MEFOXIN® (cefoxitin) MIC_{90}
B. fragilis (55)	8 mcg./ml.	16 mcg./ml.
B. ovatus (17)	16 mcg./ml.	64 mcg./ml.
B. thetaiotaomicron (13)	64 mcg./ml.	32 mcg./ml.
B. distasonis (12)	8 mcg./ml.	32 mcg./ml.
B. vulgatus (3)	8 mcg./ml.	16 mcg./ml.

* Due to susceptible strains of indicated organisms.

† Dosage in renally compromised patients may require adjustment. Please see complete prescribing information.

‡ *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Harding, G.K.M., et al.: Rev. Infect. Dis. 6 (Suppl. 1) S283-S292, 1984.
2. Fu, K.P., and Neu, H.C.: Antimicrob. Agents Chemother. 17:583-590, 1980.
3. Aldridge, K.E., et al.: Antimicrob. Agents Chemother. 26:410-413, 1984.

Please consult last page of this advertisement for brief summary of prescribing information.

Cefizox®

brand of

sterile ceftizoxime sodium and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or *PDR*. The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis; Escherichia coli; Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

***Pseudomembranous colitis* has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use.** Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, but less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGOT, SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS — CF:L14

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Contents continued from page 17A

Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis 1139
Mary M. L. Lee, Ph.D., and Ming-neng Yeh, M.D.
New York, New York

Uterine metabolism of the pregnant rabbit under chronic steady-state conditions 1146
Robert L. Johnson, M.D., Marc Gilbert, M.D., Stephen M. Block, M.D., and Frederick C. Battaglia, M.D.
Denver, Colorado

Maternal ovine placental vascular responses to adenosine 1152
Megan Landauer, M.D., Terrance M. Phernetton, B.S., and John H. G. Rankin, Ph.D.
Madison, Wisconsin

Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit 1155
Sheila M. Pride, B.Sc., M.D., Basil Ho Yuen, M.B., Ch.B., Young S. Moon, D.V.M., Ph.D., and Peter C. S. Leung, M.D.
Vancouver, British Columbia, Canada

The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat 1160
Jean Claude Veille, M.D., John M. Bissonnette, M.D., and A. Roger Hohimer, Ph.D.
Portland, Oregon

CORRESPONDENCE

Goiter in pregnancy Laszlo Hegedüs, M.D., Nini Rasmussen, M.D., and Steen Karstrup, M.D. *Herlev, Denmark* 1164

Reply Thomas J. Long, M.D., Marianne E. Felice, M.D., and Dorothy R. Hollingsworth, M.D. *Los Angeles and San Diego, California* 1164

Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein Ole Mogensen, Bent Mogensen, D.V.M., Anders Jakobsen, M.D., and Arne Sell *Aarhus and Vejle, Denmark* 1165

Measurements of delta optical density at 450 nm in SS disease George H. Nelson, Ph.D., M.D. *Augusta, Georgia* 1166

Reply Michael K. Lindsay, M.D., and Virginia R. Lupo, M.D. *Atlanta, Georgia, and Minneapolis, Minnesota* 1166

Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura Susana Meschengieser, M.D., and Maria Angela Lazzari, M.D. *Buenos Aires, Argentina* 1166

Reply James N. Martin, Jr., M.D. *Jackson, Mississippi* 1167

Contents

Mobiluncus-specific antibodies in a postoperative infection Per-Göran Larsson, M.D., Boinge Bergman, M.D., Ph.D., Carl Påhlson, B.Sc., Urban Forsum, M.D., Ph.D., and Lena Gotthardsson *Skövde and Uppsala, Sweden* 1167

Finger extension of transverse uterine incision Joseph R. Gerace, M.D. *Corona, California* 1168

Reply Radoslav Jovanovic, M.D. *New York, New York* 1169

Lupus anticoagulant and pregnancy B. Spitz, F. A. Van Assche, and J. Vermylen *Leuven, Belgium* 1169

A sonographic sign for fetal Down's syndrome Paul Defoort, M.D., and Michel Thiery, M.D., Ph.D. *Ghent, Belgium* 1169

Relative placental size in growth-retarded infants David L. Woods, M.D. *Cape Town, South Africa* 1170

Reply François Teasdale, M.D. *Montreal, Quebec, Canada* 1170

BOOKS

Books received 1171

ITEMS

Items 1172

Information for authors on pages 27A, 28A, and 29A

Index to advertisers on page 64A

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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

A LOW-DOSE TRIPHASIC OC WITH THE CONFIDENCE THAT COMES FROM EXPERIENCE



*Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptom of serious disease and discontinue oral contraceptive therapy when appropriate. These d represent only the results of selected studies. Therefore, before prescribing, the physician should read and remain alert to all data found in the complete prescribing information for the product. Please also see brief summary of prescribing information on the last page of advertisement.

A formulation with the lowest total hormonal dose in a combination OC

Phased doses of both levonorgestrel and ethinyl estradiol

Permits a decrease in total steroid dose without compromising effectiveness.

Low incidence of breakthrough bleeding

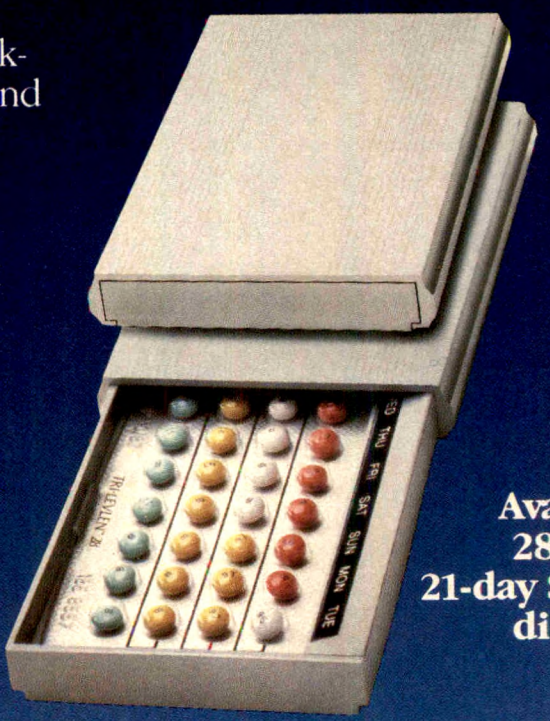
A low 5.7% overall incidence of breakthrough bleeding based on 77 U.S. and U.K. studies involving 3,546 women (35,036 total cycles).[1]

Simple first dose/first day start

The first dose is taken on the first day of menstruation. No need to count days or wait for Sunday starts.

Minimal impact on the following metabolic and hemodynamic parameters*

Lipids,[2,3] carbohydrate metabolism[2,3] and blood pressure[1] were not significantly affected in certain specific studies.



A formulation with the confidence of 170 million cycles of experience worldwide

Available in 28-day and 21-day Slidecase™ dispensers

TRI·LEVLEN™28 TRI·LEVLEN™21

BERLEX™ **Dedicated to innovation in OB/GYN medicine**

TRI·LEVLEN™ 28
TRI·LEVLEN™ 21

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. **2.** Skouby SO, Kühl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153:495-500. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513.

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The original and two good-quality copies of the manuscript and three glossy prints of illustrations are required.

Manuscripts must be typed double spaced on one side only on 22 by 28 cm (8 1/2 by 11 inch) white bond paper with 1 inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, abstract, body of text, references, legends, and tables.

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods section* describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results section* present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your observations to those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment section* state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgements. Acknowledge only persons who have made substantive contributions to the study.

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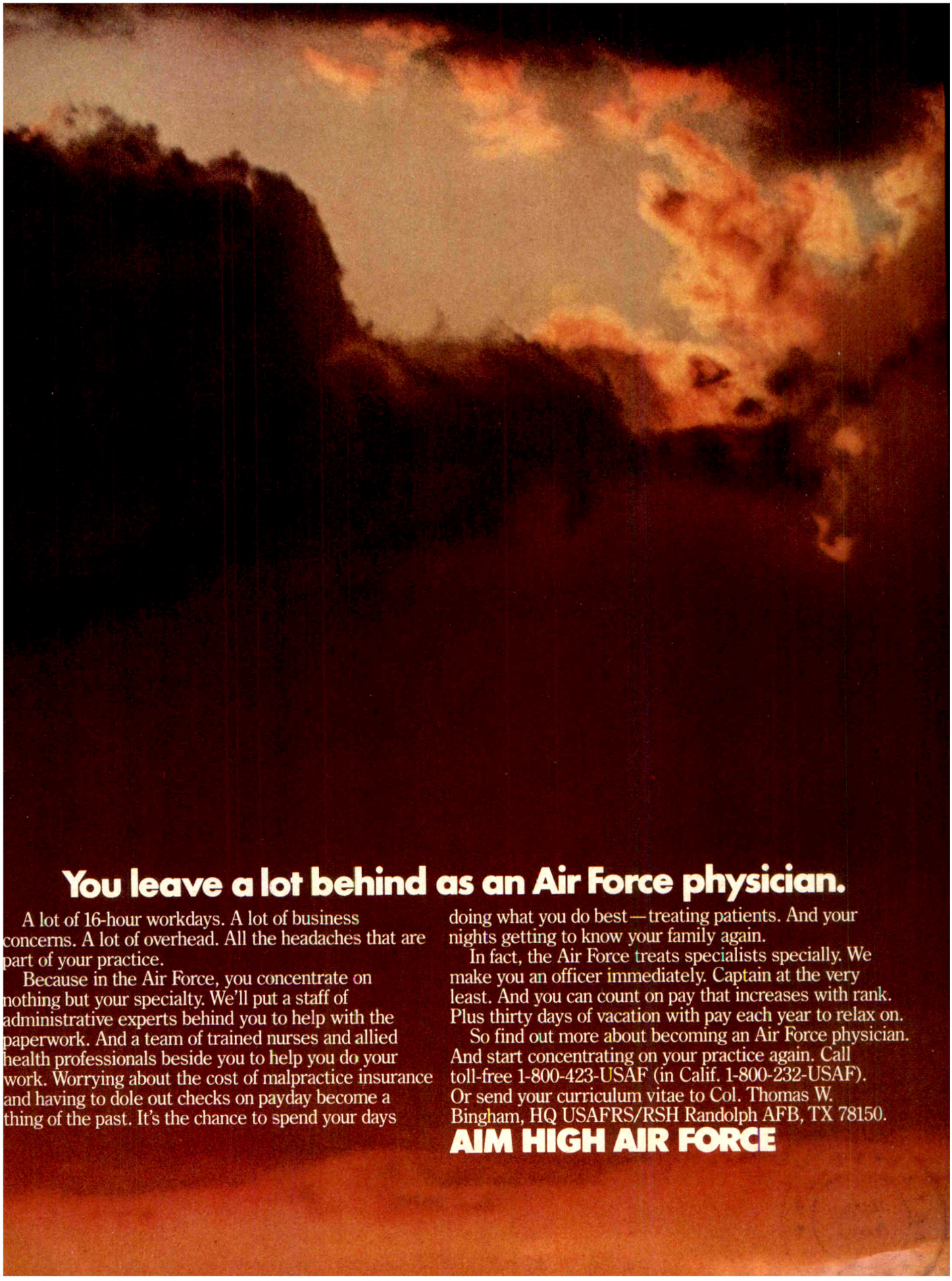
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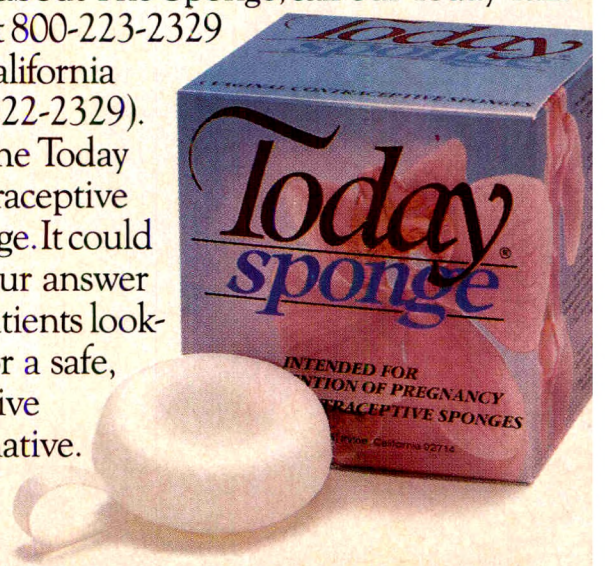
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CLINICAL SECTION

Clinical Opinion

An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis

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Group B β-hemolytic streptococcus is one of the most common causes of neonatal sepsis. Despite its relationship to neonatal morbidity and mortality, no consensus exists for an approach to its prevention. Several characteristics of the organism such as high maternal carriage rates, the intermittent nature of this carriage, and the failure of antibiotics to permanently eliminate carriage have limited the success of proposed intervention protocols. In this clinical opinion we review characteristics of the organism and previously suggested intervention protocols. Then, based on this review and an analysis of recently published data, a protocol focusing on preterm births is presented. This proposal favors intrapartum treatment of all mothers who are delivered of preterm infants and who are either carriers of group B β-hemolytic streptococci or whose carriage status is unknown. A comparison of the costs and benefits of this and other approaches is made. (AM J OBSTET GYNECOL 1986;154:973-7.)

Key words: Group B β-hemolytic streptococcus, neonatal sepsis

The contribution of group B β-hemolytic streptococcus to neonatal morbidity and mortality has long been recognized and was the subject of a National Institutes of Health workshop almost a decade ago.[1] At that forum, which brought together leading investigators, "emphasis was given . . . to the definition of priorities leading to programs for the prevention of group B beta streptococcal infection." However, despite 10 years of efforts subsequent to that meeting, no consensus exists for an approach to this problem, and as recently as 1982, the published recommendation of the Centers for Disease Control for dealing with group B β-hemolytic streptococcal sepsis in *Sexually Transmitted Diseases: Treatment Guidelines* was, in toto, "the present state of knowledge about the epidemiology of group B streptococcal disease is not sufficient to recommend routine treatment of pregnant women or their sexual partners or routine prophylaxis of their newborn."[2] Without a recommended approach, a wide variety of protocols exist which vary greatly in their scope and impact. The lack of a consensus approach to group B β-hemolytic streptococcal sepsis stands in sharp contradistinction to the management of other infectious diseases that command large investments or resources and man-hours. The disparate nature of the approaches to perinatal infections is best illustrated by the current management of herpes simplex virus in contrast to that of group B β-hemolytic streptococcal sepsis.

It is estimated that herpes simplex virus infects only 120 to 1000 neonates annually in the United States. Seventy percent of these infants die, and many survivors suffer serious sequelae. Current perinatal management involves study of weekly viral cultures in the third trimester and use of cesarean section for viral shedders at term. Though this approach has been noted to be both expensive ($53 million annually, 5000 unnecessary cesarean sections) and relatively ineffective (75% of neonates exposed to asymptomatic shedders will be missed by this protocol), it remains the de facto standard of care.[3]

For group B β-hemolytic streptococcus, on the other hand, it has been estimated that 12,000 to 15,000 newborn infants per year are infected and that the mortality rate for early-onset disease can be as high as 50% among

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premature infants.[4] Hence the number of neonatal deaths from it is several times higher than from herpes simplex virus. Yet, although many antepartum protocols for the prevention of group B β-hemolytic streptococcal sepsis have been proposed, none has been uniformly adopted because each has problems that have been clearly recognized and that may limit the benefits. Thus the lack of an ideal system has until now precluded the universal institution of any management plan, leaving the broad scope of the problem largely unaddressed.

Clearly there is a need for further research, which has been acknowledged for many years and was the focus of the National Institutes of Health workshop mentioned previously. However, although a protocol should be based on the results of a prospective randomized study, there is need for a standard approach to the prevention of group B β-hemolytic streptococcal infection until such research is completed. A study seeking to document the effectiveness of a protocol in lowering sepsis rates among preterm infants from 1% to 0.5%, for example, would have to involve a population base of over 100,000 deliveries (assuming a 9% prematurity rate) and hence probably will not be reported in the near future. Therefore, in an attempt to generate a consensus protocol that may prevent a proportion of deaths from group B β-hemolytic streptococcal disease in the interval until a more universal solution is devised (for example, vaccine), we will summarize current approaches that have been proposed as well as their shortcomings and then offer, as a clinical apinion, a management plan that would be immediately applicable and practical.

Characteristics of group B β-hemolytic streptococcal disease

Many characteristics of group B β-hemolytic streptococcal disease make it particularly insidious and difficult to eradicate. First, since up to 30% of pregnant women may be colonized, therapeutic plans directed at all group B β-hemolytic streptococcus carriers must be very broad based. Further, there are several types of carriage that can occur during pregnancy. Only 40% of colonized patients carry the organisms chronically throughout pregnancy, whereas the rest carry it either intermittently or transiently and may have a different culture status at delivery than they had antepartum. The culture characteristics of the organism also pose potential difficulties. If selective media is used, the percentage of patients who are found to be carriers will be more than double that found with standard media. These additional patients, colonized by low numbers of organisms, may not be at as significant a risk of infecting their infants. Colonization rates also vary depending on the site at which the culture is performed. There is a gradient of colonization, with the highest rates found near the rectum and progressively lower rates found toward the cervix and urethra. Infants may be infected even if the mother is colonized only in the rectum.[5]

Between 50% and 70% of women who are colonized with group B β-hemolytic streptococcal organisms will deliver infants who will also be colonized, and 1% of these colonized infants will become septic. The mortality from group B β-hemolytic streptococcal sepsis is closely linked to the gestational age of the neonate, with up to 90% of extremely premature infants but less than 5% of term infants dying. Compounding management problems is the fact that death frequently occurs despite early institution of appropriate antibiotic therapy. In addition to a higher mortality rate, an increased attack rate has been linked with prematurity. The attack rate among preterm infants is seven times that among mature infants.[4] Most neonatal deaths are a result of early-onset disease that has been associated with transmission of group B β-hemolytic streptococcal organisms from the mother's genital tract occurring either concomitantly with labor and ruptured membranes or during parturition. Thus high colonization rates, variable patterns and sites of carriage, and the relatively small percentage of colonized mothers being delivered of septic infants have made group B β-hemolytic streptococcal disease a difficult one to prevent. However, several approaches have been suggested.

Management protocols

Maternal antepartum protocol. In general, treatment schemes have been directed either at identifying and treating colonized mothers or identifying and treating colonized infants. One of the first treatment plans proposed which was directed at mothers involved culture studies of all women in the third trimester and treatment of those who were colonized to prevent them from infecting their babies at birth. The major problem with this approach was the failure of therapeutic doses of penicillin, administered to the patient and her sexual partner, to eradicate the organism, perhaps because of penicillin's decreased effectiveness in the lactamase-rich environment of the rectal flora.[6] Additionally, some patients who are not culture positive at initial testing may become culture positive at term. Finally, there may be a high rate of false negative cultures if nonselective media is used and inappropriate sites for culturing are chosen. Even if this modality of therapy were to prove effective, concern would still be raised about the number of people requiring treatment (all colonized women and their sexual partners) and the cost and risks (5% rate of adverse reactions to penicillin, 0.04% anaphylactic reaction rate) of such wide-scale antibiotic therapy.

Maternal intrapartum protocol. An alternative sug-

gestion was to identify the patient who is a carrier but then to withhold treatment until labor commences. This bypassed the problem of recolonization after earlier therapy. Further, there is evidence that intrapartum therapy, even if instituted only 2 hours before birth, can reduce or prevent neonatal sepsis. Yow et al.[7] noted no colonization among 34 infants of colonized mothers treated in labor, compared to colonization in eight of 16 infants of untreated colonized mothers. Colonized infants of treated mothers may also have lower inoculums of bacteria and lesser likelihood of sepsis. The problem with this plan is, again, that patients who are culture negative at initial screening may be positive at term and vice versa. Finally, the volume of patients who would need treatment and established infections prior to therapy remain problems.

To circumvent those problems that arise because of changes in culture status between initial cultures and labor, rapid identification techniques are being developed. Unfortunately, in the bacterial-rich vaginal environment, most techniques need long incubation periods to achieve high specificity and sensitivity. For example, Boyer et al.[8] found with use of a fluorescent antibody technique that 18 hours were needed before over 90% of carriers could be identified. Further, although the more heavily colonized mothers were identified earlier, the mothers of infants who became septic were not identified before the delivery of those neonates. Finally, even if a perfectly sensitive and specific rapid-culturing technique were available and carriers could be treated in labor, there still would be a need to treat up to 30% of all parturients.

Neonatal protocol. A report in the *Mount Sinai Journal of Medicine* gave impetus to consideration of treatment of the neonate instead of the mother.[9] That report noted that when hospital policy was switched from use of silver nitrate to intramuscular penicillin for prevention of gonococcal ophthalmia, a coincident disappearance of early-onset group B β-hemolytic streptococcal sepsis was noted. This was a retrospective report and covered some time periods (back to 1952) when the pathologic diagnosis of group B β-hemolytic streptococcal sepsis may not have been uniformly made. Although Lloyd et al.[10] subsequently reported similar findings, his study also used historic controls and the colonization rate in his treatment period was so low that one would not have expected as much morbidity as in the historic control period. Ramamurthy et al.[11] in a prospective controlled trial found no improvement with use of penicillin prophylaxis in newborn infants and noted that large fluctuations over time in colonization rates made studies of group B β-hemolytic streptococcal disease singularly inappropriate for historic controls. Siegel et al.[12] performed a 2-year prospective study in which infants delivered on alternate weeks received either intramuscular aqueous penicillin G or ocular topical tetracycline. Although the penicillin group had less group B β-hemolytic streptococcal sepsis, they had more infections with penicillin-resistant organisms. Finally, evidence that up to two thirds of infants with group B β-hemolytic streptococcal sepsis are bacteremic at birth suggests that neonatal therapy may not be the best option.[4]

Vaccines have also been considered as a potential answer to the problem of group B β-hemolytic streptococcal sepsis.[13] Mothers of infants with this sepsis are significantly less likely to have high levels of antibody than mothers of noninfected neonates. This fact has fostered the belief that immunoprophylaxis will be the ultimate solution to this dilemma. Although studies in lower animals have been encouraging, only limited success has been noted with passive or active immunization in rhesus monkeys or humans.

Proposed management

As previously mentioned, the high rates of carriage of group B β-hemolytic streptococcal disease, the frequently intermittent nature of its carriage in pregnancy, its recurrence after appropriate therapy, and the failure of antibiotics in the face of established infection in the neonate have combined to frustrate attempts to safely eradicate neonatal disease. Protocols aimed at all carriers of disease are extremely treatment-inefficient, whereas the more focused protocols that are directed at infected neonates are usually instituted too late to be successful. To be more efficient and more timely, the focus of intervention should remain on the mother yet be narrowed from being on all carriers to high-risk carriers only. After reviewing their data at the Medical Center of Vermont, Pasnick et al.[14] suggested that "an alternative approach to these problems would be the identification of risk factors for early onset disease and treatment of only those . . . at high risk." A major step in the direction of developing a focused maternal intervention protocol is based on the work of Boyer and his colleagues.[4, 5, 15] After performing exhaustive studies on maternal-fetal transmission of group B β-hemolytic streptococcal organisms,[5] carriage patterns,[15] and the chemotherapeutic impact of intrapartum antibiotics,[16] they proposed a plan based on antepartum screening and intrapartum treatment. Their proposal would result in the treatment of only 3% of all patients by aiming solely at patients who are both colonized (16.7% of their population) and who have risk factors for an increased attack rate (18.3% of their population—those with low birth weight, rupture of membranes at >18 hours, or intrapartum fever). The data of Pasnick et al.[14] noted group B β-hemolytic streptococcal morbidity in the same subgroups. Boyer subsequently documented the efficacy of this approach in a study of intrapartum use

of ampicillin. Although their work represents an impressive body of data in support of intrapartum treatment of risk patients, several concerns about the practicality of routine institution of this approach remain.

The first problem with their specific plan is that although it narrows the focus of therapy it still requires the institution of universal screening, which reduces the chances of its being widely accepted. Costs would be high, since their results are based on screening all patients with both vaginal and rectal cultures and use of selective media. Based on current costs for cultures and therapy, projected to involve >3.6 million births annually, the cost would be $108 million for universal screening and $2 million for treatment with ampicillin for 3% of all women. The second problem is that therapy is based on results of screening performed at 26 to 28 weeks. In indigent populations, which have higher prematurity rates and higher attack rates for group B β-hemolytic streptococcal disease, many patients will present in the third trimester or in labor for initial care and will not have culture results available. Also, carriage status may change between the time of culture and labor. Boyer found that the overall predictive value of a positive prenatal culture was 67.2% and that 8.5% of patients who were initially culture negative became culture positive by term. Further, the number of patients becoming positive after negative prenatal screens would be substantially higher if rectal cultures were not performed on all prenatal patients. Again, the efforts and costs involved in instituting vaginal and rectal cultures on all patients makes adaptation of this protocol unlikely. Finally, if screening is performed at an average of 27 weeks and if we assume that all results are available at the patient's subsequent visit, then a large percentage of patients whose neonates will die with group B β-hemolytic streptococcal sepsis will be born before results are available (in Boyer's study, one half of all deaths were among infants under 1000 gm). If cultures are done earlier to surmount that problem, then the predictive abilities of prenatal screens, cited above, would be further compromised. Despite these problems Boyer's work represents an important step toward developing a practical approach to this major problem.

The approach that we propose in this clinical opinion would result in a slight increase in the number of parturients receiving antibiotics in labor while substantially reducing the number of patients needing screening, hence minimizing the logistic complexities that universal screening entails. Among the groups at high risk for sepsis as delineated by Boyer, we would focus on preterm infants. Approximately 93% (15 of 16 in Boyer's study) of neonatal deaths associated with group B β-hemolytic streptococcal early-onset disease occurred in that group. Patients with chorioamnionitis, one of the other risk groups, routinely receive antibiotics independent of group B β-hemolytic streptococcal culture status. Attempting to treat the final risk group, all culture-positive term patients with ruptured membranes >18 hours would reduce the total number of deaths associated with group B β-hemolytic streptococcal disease only slightly while requiring a tremendous increase in the need to obtain cultures as well as timely results. Further, since sepsis in their study occurred at or beyond 18 hours of the rupture of the membranes, it would be necessary to start therapy earlier in the course of labor to prevent morbidity and hence would necessitate therapy of a markedly increased percentage of patients once the focus expanded from patients with rupture >18 hours to those patients who may eventually have rupture >18 hours.

We suggest that for all patients admitted in preterm labor and/or with preterm premature rupture of membranes (<37 weeks' gestation), cultures for group B β-hemolytic streptococcal disease be obtained. If labor is successfully inhibited with tocolysis, antibiotic therapy can be withheld pending culture results. All culture-positive patients should be treated with ampicillin (2 gm every 6 hours for 24 hours, then switched to 500 mg every 6 hours orally for 2 weeks, and then recultured; erythromycin is used for the penicillin-allergic patient). If the patient's labor is not successfully inhibited and the culture result is pending, then antibiotic therapy is begun. If a patient being managed conservatively with preterm ruptured membranes goes into labor (assuming tocolysis is not used), antibiotics are given if the culture is pending (2 gm intravenously as soon as possible, then 2 gm every 6 hours). Thus all therapy and culturing is reserved for the approximately 8.9% of the population who deliver prematurely and who account for over 90% of group B β-hemolytic streptococci–associated deaths. The actual number receiving therapy may even be less, depending on the number of patients whose labor is successfully inhibited while culture results are pending. Although a large number of culture-negative patients will receive unnecessary antibiotics because of unavailable culture results, at the most this number represents approximately 7.5% of all patients (prematurity rate, 8.9% times the culture-negative rate, or 83.3% in Boyer's work) which is not markedly different from the approximately 6% of patients who would receive unnecessary therapy when all risk groups that are culture positive at 26 to 28 weeks are treated in labor (18.3%, total of all risk groups, times 32.8%, patients positive at prenatal visit but not positive during delivery). The slightly increased need for therapy is balanced against a substantial decrease in the number of patients requiring cultures and the number of patients not receiving needed therapy,

because although their culture status was positive during delivery, it was negative at initial screening (8.5% of risk patients). Perhaps most critically, this system would be simple to implement. Finally, if simple, accurate, rapid diagnostic tests for group B β-hemolytic streptococcal disease become readily available, then the number of patients receiving unnecessary therapy because culture results are not available would markedly decline. If the protocol is instituted, it is imperative that hospitals using it keep a careful record of all neonatal deaths attributable to sepsis so that failures related to parochial resistance patterns, established infection, or other problems are quickly detected.

An important question that remains is the safety of waiting for culture results for the preterm patient with ruptured membranes being managed conservatively. Unfortunately, the risk of ascending infection with group B β-hemolytic streptococcus during the time that culture results are pending is unknown. If rapid screening were available, risks could be minimized, since antibiotics could be instituted within hours of rupture. Without rapid screening it is necessary to consider initiating use of prophylactic antibiotics on admission for patients with premature rupture of membranes. However, the risk/benefits ratio of that approach is difficult to assess pending the results of studies currently under way.

This paper does not address the question of group B β-hemolytic streptococcal disease as a cause of preterm labor or premature rupture of membranes. Various authors have reported divergent results on that subject[4, 16] and the potential benefit of treating group B β-hemolytic streptococcal disease to prevent preterm labor or premature rupture of membranes has not yet been demonstrated. Unless that can be clearly shown, we believe that focusing on patients with preterm infants and treating either those with culture results positive for group B β-hemolytic streptococcal disease or those whose culture status is unknown is a reasonable protocol with which to begin to address a neonatal disease that far surpasses herpes simplex virus as a cause of perinatal mortality. As for concern about obscuring the diagnosis of neonatal disease among infants born to antibiotic-treated mothers, we concur with Boyer's statement that "when there is a substantial and well-defined risk of intrauterine infection, the potential benefit of intrapartum prophylaxis for the newborn infant more than compensates for increased uncertainty in postnatal diagnosis." Finally, although we also agree that "the possibility of selecting resistant flora by blanket use of prophylaxis remains a disturbing risk" and that "additional controlled data documenting safety and efficacy of selected intrapartum chemoprophylaxis in preventing invasive disease will be required before a firm recommendation can be made," it is our clinical opinion that in this particular population, our therapeutic strategy is warranted while the aforementioned studies are ongoing.

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Perinatal events and cerebral palsy

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This review highlights recent studies that refute the following hypothesis on the genesis of cerebral palsy: The risk factors that cause death are also risk factors for brain damage resulting in cerebral palsy, if they occur at lower intensity, less frequently, or for a shorter duration. Untested, unproved, and invalid theories that emerged in the 1950s stimulated the assembly of much data that are at odds with the notion that the pathways of causation for cerebral palsy and perinatal mortality are identical. (AM J OBSTET GYNECOL 1986;154:978-81.)

Key words: Cerebral palsy, brain damage, birth injury, asphyxia neonatorum, perinatal morbidity

More than a century ago Little[1] associated a group of childhood "deformities" and mental states with asphyxia neonatorum, abnormal parturition, difficult labors, and premature birth. Significant efforts to further link these events to the cause of abnormal human neurological development have included refined epidemiologic studies and experiments with animal models. Even so, the pregnancy or labor conditions that will predictably result in defects, such as cerebral palsy, are not established.

In 1951, Lillienfeld and Parkhurst[2] offered a unifying hypothesis of common causation for both perinatal mortality and cerebral palsy. This was based on their analysis of the initial data from the New York Crippled Children's Program. They postulated the existence of a "continuum of reproductive wastage" with lethal and sublethal manifestations. The lethal component included abortions, stillbirths, and neonatal deaths. The sublethal component included cerebral palsy and possibly other disabilities. From this analysis, the authors suggested that the preventive aspect of a cerebral palsy program should be placed within the general scope of a maternal health program. Thus they extended the hypothesis to include a continuum of causation and prevention, implying that obstetric innovations that brought about improvements in perinatal mortality would also reduce the frequency of occurrence of cerebral palsy and related conditions. Subsequently, the obstetric and pediatric literature has accepted the concept of a common causation of perinatal mortality and cerebral palsy to the point that it is essentially dogma.

It should now be pointed out that much data are currently available that are at odds with the notion that the pathways of causation for cerebral palsy and perinatal mortality are identical. In fact, there may be only a few common factors among many that are disparate. This review will highlight recent studies which refute the following hypothesis on the genesis of cerebral palsy: The risk factors that cause death are also risk factors for brain damage resulting in cerebral palsy, if they occur at lower intensity, less frequently, or for a shorter duration.

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Animal models of cerebral palsy

Brain damage induced by either chronic or acute hypoxia in rhesus monkeys demonstrates specificity regarding the site of damage and the pattern of the abnormality according to the time course of induced hypoxia.[3] It was at first surprising and later striking that attempts to regularly produce a clinical picture like cerebral palsy in these animals was not successful. In most cases, the fetuses either survived experimental asphyxia and were neurologically intact or they died.

An alternative strategy by Clapp et al.[4] involves a search for experimental conditions in the fetal sheep model to induce brain damage without producing hypoxia or acidosis. Two sets of perturbations in this animal model were shown to cause histologic damage in cortical white matter. The first involved partial (20% to 80%), intermittent (10 seconds on, 10 seconds off) umbilical cord occlusion for 1 of every 3 minutes for a total duration of 2 hours during late gestation. In a second group of animals growth retardation of the fetus was produced by embolization of the uterine circulation, a previously described procedure that does not cause brain damage. Subsequently, however, the brain was indeed damaged when mild hyperglycemia was produced in the fetuses by raising the blood glucose from about 20 to 40 mg/100 ml for 4 hours in late gestation. Neither of these experimental conditions resulted in fetal deaths or even evidence of significant fetal hypoxia or acidosis.

Recent studies on intracerebral hemorrhage in both term and preterm human fetuses have also focused on

the dominant role of fluctuating cerebral blood flow in determining the incidence and severity of hemorrhage.[5, 6] Especially the more severe hemorrhages are associated with subsequent impaired neurological development. These studies suggest that transient shifts in blood flow, while generally not linked to fetal or neonatal death, may contribute substantially to intracranial hemorrhage and brain damage.

Epidemiology of cerebral palsy and perinatal mortality

The incidence of cerebral palsy in developed countries is about 1.5 to 2.5 per 1000. Comparisons between countries show no correlation with the prevailing perinatal mortality rate.[7] Differences in the absolute incidence are often attributable to the definitions and study criteria used rather than to the populations. The great improvement in perinatal mortality of the last 25 years has been accompanied by an essentially unchanged incidence of cerebral palsy. During the course of the United States Collaborative Perinatal Project (1959 to 1966) and also through the course of a Swedish study extending from 1958 through 1976, there were substantial reductions in perinatal mortality but a conspicuous lack of parallel decreases in cerebral palsy.[7, 8] A somewhat different trend was seen in the data from the Western Australian cerebral palsy register.[9] From 1961 through 1975, there was a downward trend in cerebral palsy incidences for heavier infants (>2500 gm) whereas the rate remained steady among low birth weight infants. Although this phenomenon has not been confirmed in other reports, recent improvements in survival of very low birth weight infants could make it difficult to identify a falling incidence of cerebral palsy occurring only in larger infants.

The magnitude of the confounding effect of increased and increasing numbers of preterm deliveries can be estimated with the recently published data of Shennan et al.[10] and Milligan et al.[11] from the Regional Perinatal Unit, Women's College Hospital, Toronto, Ontario, Canada. For births occurring in 1979 through 1982, there were 383 live births with gestations between 26 and 30 weeks. At 1 year there were 349 surviving children, of whom 27 (about 8%) had cerebral palsy. Twelve of these 27 children had either severe motor disability or mental retardation in addition to motor disability. Deliveries occurring before completion of 30 weeks' gestation constitute about 0.5% of total live births.* Cerebral palsy in 8% of these very preterm infants would contribute 0.4 per 1000 to the overall population incidence of cerebral palsy. In the last decade several tertiary units have shown a doubling of survival for infants born at this early gestational age. In the example above, half of the cerebral palsy cases in deliveries at 26 to 30 weeks' gestation may be attributed to the recently increased survival of very preterm babies, and this increment would constitute an additional 0.2 per 1000 to the overall population incidence. This potential confounding effect could constitute about 10% of the prevailing incidence. If the vast improvement in overall perinatal mortality during recent years were accompanied by similar reductions in cerebral palsy, this small influence due to increased survival of very small infants should not obscure the expected decreased incidence of cerebral palsy.

Another observation among children followed up until age 7 in the United States Collaborative Perinatal Project was the higher incidence of cerebral palsy in white children (6.0 per 1000) than in black children (4.3 per 1000).[7] These rates are statistically different and there is obvious discordance with preterm births and perinatal mortality, both of which are more frequent in black children.

Poor correlation between asphyxia and occurrence of cerebral palsy

The Collaborative Perinatal Project data show a relationship between low Apgar scores and cerebral palsy only when there are very low Apgar scores for prolonged periods.[12] Table I shows that for infants with Apgar scores of ≤3 beyond 5 minutes, there is a linear relationship between the percentage of survivors with cerebral palsy (among infants >2500 gm) and duration of low score. However, in the same data it is evident that a clear majority (73%) of children who developed cerebral palsy had 5-minute Apgar scores of ≥7. Thus the 5-minute Apgar score is neither sensitive nor specific in predicting cerebral palsy.

A major argument for use of fetal heart rate monitoring in labor has been the ability to recognize fetal distress patterns associated with intrapartum and neonatal mortality. A similar correlation has not been made with postpartum neurological or brain injury. Painter et al.[13] have followed the neurological development of a group of children who had late or severe variable fetal heart rate decelerations during labor. Forty-five of the original 50 patients were examined at age 6 to 9 years. Detailed neurological examinations showed that all were normal except for one child with a genetic syndrome who demonstrated persistent late decelerations in labor.

Similarly, the physical and mental development in infants delivered after a positive contraction stress test has been reported by Crane et al.[14] Although the 12 subjects compose a small sample and the follow-up ranges from only 5.5 months to 4.75 years, the results are encouraging. No major neurological abnormalities

*California Birth Certificate Data, 1983. Personal communication, Ronald L. Williams, Community and Organization Research Institute, University of California Santa Barbara, Santa Barbara, California.

Table I. Collaborative Perinatal Project data on Apgar scores and cerebral palsy by birth weight

	<2500 gm		>2500 gm	
	Survivors with cerebral palsy (%)	*No. of cerebral palsy cases*	*Survivors with cerebral palsy (%)*	*No. of cerebral palsy cases*
1 min Apgar score 7-10	0.6	13	0.2	53
1 min Apgar score 0-3	2.9	9	1.5	22
5 min Apgar score 0-3	6.7	5	4.7	13*
10 min Apgar score 0-3	3.7	1	16.7	11
15 min Apgar score 0-3	0	0	36.0	9
20 min Apgar score 0-3	0	0	57.1	8

*A total of 73% of individuals with cerebral palsy had 5-minute Apgar scores of ≥7.

were detected and 10 of the 12 displayed normal mental and motor function, as determined by the Denver Development Screening Test, Bayley Scales of Infant Development, and Wechsler Preschool and Primary Scale of Intelligence. The two exceptions were infants with additional serious neonatal complications who scored poorly only on the Bayley test.

Another report on severe acidemia at birth in The Netherlands provides the follow-up neurological examinations from 2.5 to 5 years on 24 normally grown infants delivered at term who had no birth trauma or birth defects.[15] The umbilical blood pH of all infants was ≤7.0. Major neurological abnormalities were not found in this group, but three infants were classified as neurologically "suspect" because of mild to moderate hypotonia. A comparison group of 115 nonacidemic infants who met the same selection criteria also had follow-up examinations that yielded 10.5% neurologically "suspect" or abnormal children. They concluded that acidemia itself is not a major cause or predictor of later neurological abnormalities in normally grown term infants.

Evidence for multifactoral causation of cerebral palsy and noncorrelation of cerebral palsy with causation of perinatal morality

The analysis by Lillienfeld and Parkhurst,[2] which led them to formally state the "continuum of reproductive wastage" hypothesis, focused on the fact that in pregnancies resulting in infant loss and producing children with cerebral palsy, some of the same pregnancy complications occurred. Complications having similarly increased frequencies in these two adverse outcome groups included placenta previa, breech and other malpresentations, and dystocia.

However, there were many complications that had markedly discrepant frequencies between pregnancies with perinatal mortality and pregnancies resulting in birth of a child with cerebral palsy. Premature separation of the placenta, complications of the umbilical cord, bleeding during pregnancy, and toxemia were much more closely associated with infant loss. Deviations of the bony pelvis were more frequently associated with the delivery of an infant with cerebral palsy. Their data could clearly form the basis for a very different conclusion than the one they proposed.

Their data and numerous subsequent studies show that many and diverse conditions and perinatal events occur with increased frequency in pregnancies with a disappointing neonatal outcome. One might have argued that these two categories of adverse outcomes, cerebral palsy and perinatal death, are each unique in regard to their many associated and possibly causative conditions. Among the linked causes for each, there may be a small common domain.

The analysis of perinatal factors associated with death or neurological handicap in preterm infants by Shennan et al.[10] makes this point even more clearly. The clinical events for infants who died were compared to those for surviving normal infants who were born at the same gestational age (between 26 and 30 weeks). Mothers of infants who died were less likely to have been in preterm labor and to have received betamethasone. The fetuses who died were less likely to have had a normal heart rate pattern and were more likely to be male. Statistically significant correlations with death in the newborn period included low 5-minute Apgar score, low venous cord pH, cord blood base deficit, low blood pressure in the first 72 hours of life, and occurrence of respiratory distress syndrome. In addition there was a direct relationship between gestational age at birth and probability of survival.

In marked contrast, none of the 18 obstetric factors analyzed occurred at higher frequency in women who were delivered of a child with a subsequent major neurological handicap. Among the 23 neonatal factors studied, handicapped infants were statistically different from control infants only in the maximum oxygen concentration and respiratory rate required. The only common factor associated at increased frequency with both death and handicap in this study was intraventricular hemorrhage in the newborn period. The conclusion of this study is clearly stated by the authors: ". . . contrary to our traditional understanding, there is

a difference between the factors leading to death of the newborn infant and those leading to damage in survivors at the same gestational age."[10]

Perinatal mortality rates have greatly improved since obstetricians implemented preventive approaches that utilize the current diagnostic tests available for fetal growth and well-being in the antepartum period in addition to fetal heart rate monitoring during labor. Obstetricians have assumed that the strategy of applying these same diagnostic innovations could lead to marked improvements in the occurrence of brain damage among surviving infants. However, the prevention of neurological damage and the anticipated decline in cerebral palsy have not occurred.

Prematurity and asphyxia are definitely associated with cerebral palsy. What we presently lack is an understanding of the underlying conditions responsible for brain injury when the infant is born prematurely or when asphyxia occurs despite our best efforts. In this regard, preventive programs may remain unsuccessful until the causation of cerebral palsy is more fully understood. While we have learned much about causation and prevention of perinatal mortality, very little has been established about the causation and prevention of cerebral palsy.

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Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear

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Human papillomaviruses have previously been identified by molecular hybridization in the majority of dysplastic and cancerous lesions of the cervix. Since human papillomavirus types 16 and 18 have been strongly associated with cervical cancer, the identification of patients infected with these specific human papillomavirus types may provide useful prognostic information. We have developed a painless, noninvasive cervicovaginal lavage technique to collect exfoliated cervicovaginal cells, which can be reliably analyzed for the presence of human papillomavirus deoxyribonucleic acid by Southern blot analysis with the use of deoxyribonucleic acid cloned from human papillomaviruses 6, 11, 16, and 18. In a prospective study of 60 women referred to a colposcopy clinic for evaluation of abnormal Papanicolaou smears, we have detected human papillomavirus deoxyribonucleic acid in 16 of 17 (94%) women with a Class III (dysplasia) or IV (carcinoma in situ) Papanicolaou smear, five of 11 (45%) women with a Class II (atypical) Papanicolaou smear, and 10 of 34 (29%) women with a normal Papanicolaou smear. Detection of human papillomavirus deoxyribonucleic acid in cervicovaginal cells was indicative of a dysplastic cervical lesion in 19 of 20 (95%) patients irrespective of Papanicolaou smear results. We conclude that human papillomavirus deoxyribonucleic acid analysis in cervicovaginal cells is a sensitive method to detect dysplastic lesions of the cervix and may be useful in identifying patients with specific types of human papillomavirus infection, who are at risk to develop cervical cancer. (Am J Obstet Gynecol 1986;154:982-9.)

Key words: HPV, cervical dysplasia, Pap smear

The human papillomaviruses are a heterogeneous group of small double-stranded circular deoxyribonucleic acid (DNA) viruses that infect the squamous epithelium of man and produce a variety of proliferative diseases (reviewed in references 1 and 2). Recent advances in recombinant DNA technology have allowed the molecular cloning of more than 30 different types of human papillomaviruses. With the use of DNA hybridization, at least four different human papillomavirus types, 6, 11, 16, and 18, have been identified in tissue originating from cervical and genital lesions.[2, 3] HPV types 6 and 11 are frequently identified in benign genital and cervical warts, whereas human papillomavirus types 16 and 18 are the predominant types found in cervical and genital carcinomas.[2, 3] Human papillomavirus type 16 DNA has also been identified in high-grade dysplastic lesions of the cervix.[4] Not only are human papillomaviruses 16 and 18 DNA sequences present in cervical cancer cells, but they are also transcriptionally active both in cultured cervical cancer cells and in fresh tumor tissue.[5] DNA sequences of human papillomaviruses types 16 and 18 are frequently integrated into the genomes of cervical cancer cells, whereas they exist as episomal plasmids in most premalignant cervical lesions.[2, 5] Thus specific types of human papillomaviruses are found in both invasive and preinvasive lesions of the cervix. Furthermore, accu-

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mulating evidence is incriminating specific types of these viruses as potential oncogenic agents in cervical cancer.

Exfoliative cervicovaginal cytologic (Papanicolaou) testing is an effective method for detecting dysplastic (cervical intraepithelial neoplasia) and malignant cervical cells. The technique has become widely used as a cancer screening test. Unfortunately, the biologic behavior of precancerous lesions from which cells exfoliate cannot be predicted on the basis of morphologic features alone.[6] This clinical dilemma is related to the fact that although a large proportion of cervical intraepithelial neoplastic lesions may take many years to progress, some may fail to progress or may even regress.[6] Moreover, other cervical intraepithelial neoplasia can be aggressive and rapidly progress to invasive carcinoma. Women with rapidly progressive lesions are rarely identified by periodic Papanicolaou smears before the development of invasive cancer.[7-9] While large-scale cervical cancer screening programs have reduced deaths from invasive cancer, there are a significant number of women who have undetected lesions despite regular cytologic examination.[7,8] Moreover, a single Papanicolaou smear from a patient with a cervical lesion may be negative due either to inadequate sampling of exfoliated cells or to incorrect cytologic interpretation (reviewed in reference 10). The false negative rate for a single Papanicolaou smear in women with histologically confirmed lesions can be as high as 50%.[10]

If, in fact, cervical cancer develops with greater frequency in women infected with specific human papillomavirus types, then the early detection of such infections could serve to identify women at risk for developing an invasive lesion. To investigate the hypothesis that infection with specific human papillomavirus types is related to the oncogenesis of cervical cancer, we have initiated a prospective study to detect human papillomavirus DNA in cervicovaginal cells from women attending a colposcopy clinic. The observation that dysplastic and condylomatous epithelial cells exfoliate readily suggested the possibility that the use of a lavage technique to collect exfoliated cells coupled with molecular hybridization for human papillomavirus DNA might provide a sensitive viral screening test.[11] We have developed a simple, noninvasive, and painless method to reliably sample cervicovaginal epithelial cells whereby human papillomavirus DNA can be detected by molecular hybridization. Since human papillomavirus infection cannot be documented by culture of virus or by serologic testing, we have compared the results of human papillomavirus DNA analysis of exfoliated cervicovaginal cells obtained by cervicovaginal lavage with the direct analysis of human papillomavirus DNA in cervical biopsy specimens. In this report, we present the results demonstrating (1) the sensitivity of detecting human papillomavirus DNA in cervicovaginal cells, (2) the general applicability of this method as a screening procedure to detect infection by specific types of human papillomavirus, and (3) the comparison of viral DNA findings to the results of cervical cytologic testing in simultaneously obtained specimens. In addition, we present evidence that the presence of human papillomavirus DNA correlates well with the presence of a dysplastic cervical lesion. The histopathologic, immunocytochemical, and ultrastructural changes induced in cervical epithelial cells by specific types of human papillomaviruses are described elsewhere.[12]

Methods

Specimens. All specimens were obtained from women attending a colposcopy clinic at the Bronx Municipal Hospital Center. New patients were referred for colposcopic evaluation after either two consecutive Class II (atypical), one Class III (suspicious), or one Class IV (positive) Papanicolaou smear. Other patients were seen for routine follow-up visits for a past history of cervical dysplasia, some of these women had been treated years ago and are considered to have been cured. A routine Papanicolaou smear was obtained and was followed by the collection of cervicovaginal fluid washings. This was accomplished by swabbing the cervix with normal saline solution and the application of a 5% acetic acid solution. Thereafter, the cervix was lavaged with approximately 7 to 8 ml of saline solution and the washings were aspirated from the posterior vaginal fornix. Colposcopic examination was performed on all patients and biopsies of abnormal-appearing areas were obtained under colposcopic vision. Adjoining biopsy specimens were sent for histopathologic diagosis and DNA analysis, respectively. Biopsy specimens for histologic examination were embedded in paraffin and stained with hematoxylin and eosin. Grading of dysplasia was done according to the World Health Organization criteria for mild, moderate, and severe dysplasia and carcinoma in situ.[13] Papanicolaou smears were evaluated for abnormal cells and reported as Class I (negative), Class II (atypical), Class III (suspicious), or Class IV (positive) by routine criteria.[11]

Molecular hybridization. Human papillomavirus types 6, 11, 16, and 18 cloned in pBR322 were generously provided by Dr. L. Gissmann, Freiburg, Federal Republic of Germany. Human papillomavirus recombinant clones were amplified and purified by standard methods.[14] The human papillomavirus DNA insert was purified from the plasmid DNA by electroelution and radiolabeled by primer extension.[14,15]

Biopsy tissue (approximately 50 mg) was placed directly in cervical biopsy solution (50 mmol/L Tris, pH 8.6, 25 mmol/L ethylenediaminetetraacetic acid (EDTA), 500 μg/ml proteinase K, and 0.2% sodium

Table I. Human papillomavirus DNA analysis of cervical biopsy specimens and exfoliated cervicovaginal cells taken at the same examination

HPV DNA in cervicovaginal cells	*Cervical biopsy HPV DNA*	
	Positive	*Negative*
Positive	14	6
Negative	0	9

HPV = Human papillomavirus.

dodecyl sulfate), heated to 60° C for 1 to 2 hours, and rotated overnight at 42° C. DNA was purified by two phenol extractions and two chloroform–isoamyl alcohol extractions. The salt concentration was adjusted to 0.2 mol/L potassium acetate and the DNA was precipitated by adding two volumes of cold absolute ethanol. The DNA was concentrated by centrifugation at 11,000 rpm for 20 minutes at 4° C, dried, and resuspended in 25 μl of TE buffer (10 mol/L Tris, pH 7.4, and 0.1 mol/L EDTA).

Exfoliated cervicovaginal epithelial cells were separated from the lavage fluid by centrifugation and resuspended in cervical biopsy solution. DNA was then isolated as described above for cervical biopsy tissue.

To detect human papillomavirus DNA, 5 to 10 μg of DNA from cervical biopsy tissue or cervicovaginal cells was digested overight with Bam HI, Eco RI, or Pst I according to manufacturer's specifications and the resultant DNA fragments were separated in 0.7% agarose slab gels by electrophoresis. The gels were stained with ethidium bromide and photographed under ultraviolet light, and the DNA was denatured and neutralized, then transferred to Zetabind membranes (Bio-Rad) according to manufacturer's specifications. The Zetabind membranes were prehybridized in 5X SCC (1X SCC = 0.15 mol/L sodium chloride and 0.015 mol/L sodium citrate), 20% deionized formamide, 50 mmol/L sodium phosphate (pH 6.5), 500 μg/ml of alkali-denatured salmon testis DNA, and 0.2% Ficoll, 0.2% polyvinylpyrrolidone, and 0.2% bovine serum albumin, at 42° C for a minimum of 8 hours. All hybridizations were performed under conditions of low stringency (to detect related human papillomavirus types): 5X SSC, 20% formamide, 50 mmol/L sodium phosphate, 250 μg/ml of salmon testis DNA, and 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin, with 5 to 10 × 10^6 cpm/ml of radiolabeled human papillomavirus DNA, specific activity 4 to 8 × 10^8 cpm/μg of DNA, for 24 to 36 hours at 42° C. After hybridization, filters were washed at 45° C under conditions of low stringency (minimum of 0.5 mol/L sodium chloride) as previously described,[15] kept moist, exposed for 24 to 48 hours at −70° C, rewashed under conditions of high stringency (0.1X SSC and 0.1% sodium dodecyl sulfate) at 60° C, kept moist, exposed for 1 to 7 days. In order to reuse membranes for subsequent hybridization with different probes, membranes were either treated with alkali according to manufacturer's suggestion or boiled in 0.1X SSPE (1X SSPE = 18 mmol/L sodium chloride, 10 mmol/L sodium phosphate, and 1 mmol/L EDTA/0.5% sodium dodecyl sulfate) for 20 minutes to remove radioactive probe. Membranes were routinely reexposed to assure that the probe had been dislodged. In addition, all filters were subsequently hybridized with radiolabeled pBR322 to ascertain that the human papillomavirus signal was in fact derived from human papillomavirus sequences. No hybridization bands were detected with radiolabeled pBR322 DNA.

Determination of human papillomavirus type was ascertained by a combination of (1) comparison of hybridization signal intensity of human papillomavirus DNA in samples with different human papillomavirus type probes and (2) restriction enzyme cleavage patterns. All samples were analyzed for human papillomavirus type without knowledge of histologic or cytologic findings.

Results

To determine the specificity and sensitivity of detecting human papillomavirus DNA in exfoliated cervicovaginal cells isolated from cervicovaginal lavage, we compared the DNA hybridization results obtained from cervicovaginal cells with those obtained from colposcopically directed biopsy tissue specimens. Sixty women were entered into the study; 29 had human papillomavirus DNA analysis of both cervical biopsy specimens and cervicovaginal cells taken during the same examination. The results of human papillomavirus DNA analysis of biopsy specimens and cervicovaginal cells from those 29 patients are shown in Table I. Twenty of the 29 (69%) patients had positive test results for human papillomavirus DNA in either cervicovaginal cells or cervicovaginal cells and cervical biopsy specimens. Fourteen (48%) patients had human papillomavirus DNA in both biopsy specimens and cervicovaginal cells; six (21%) patients had human papillomaviruses DNA detectable in cervicovaginal cells but not in the cervical biopsy specimens; and nine (31%) patients had no detectable human papillomavirus DNA in either biopsy specimens or cervicovaginal cells. In no case was DNA found in a biopsy specimen but not in cervicovaginal cells.

An example of an experiment determining the presence and type of human papillomavirus DNA in cervical biopsy specimens and cervicovaginal cells is shown in Fig. 1. DNA isolated from specimens obtained from two patients was digested with Bam HI, and Southern

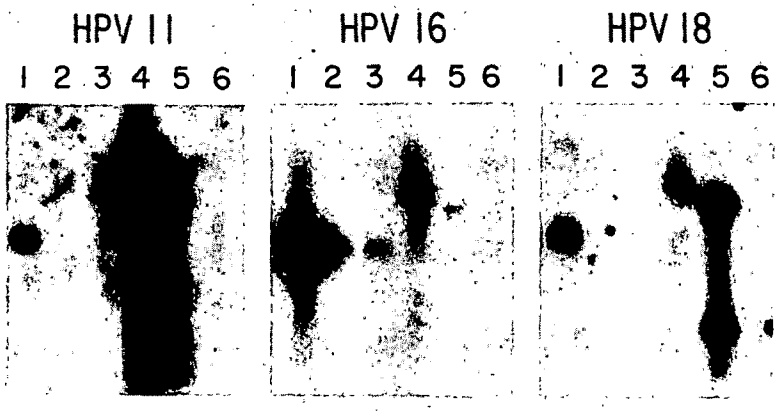


Fig. 1. Comparison and typing of human papillomavirus DNA in cervical biopsy specimens and washings from two patients. DNA isolated from two cervical biopsy specimens (lanes *1* and *2*) and the exfoliated cervicovaginal cells from lavage (lane *3*) of Patient B. G. and from cervical biopsy (lane *4*) and cervicovaginal (lane *5*) of Patient D. B. were digested with Bam HI, and a Southern blot experiment was done. The same membrane was sequentially hybridized with four different types of radiolabeled human papillomavirus insert deoxyribonucleic acid (only three are shown). The three panels show the results after hybridization with human papillomavirus types 11, 16, and 18 followed by stringent washing. Lane *6* contained radiolabeled lambda Hind III markers.

blot experiments were performed. The same membrane was sequentially hybridized with the four different human papillomavirus radiolabeled probes as described in Methods. The three panels show the Southern blot results after hybridization with human papillomavirus types 11, 16, and 18 after stringent washing. The DNA from biopsy specimen A of Patient B. G. (lane *1*) hybridized with all human papillomavirus types but showed the strongest hybridization with type 16, whereas the DNA from biopsy specimen B and the cervicovaginal cells of Patient B. G. (lanes *2* and *3*, respectively) showed only hybridization to type 16. Biopsy specimen A of Patient B. G. (lane *1*) hybridized with types 11 and 18 since different human papillomaviruses contain varying degrees of homologous sequences that can cross hybridize when a large quantity of human papillomavirus DNA is present in a sample specimen (see lane *4* also). Therefore, the three specimens from Patient B. G. (lanes *1* to *3*) contained human papillomavirus type 16. The DNA from the biopsy specimen of Patient D. B. (lane *4*) showed the strongest hybridization with human papillomavirus type 11, whereas the DNA from cervicovaginal cells (lane *5*) displayed two patterns of hybridization. With human papillomavirus type 11 *(left panel)* a single band similar in size to but less intense than that in lane *4* was present, and with human papillomavirus type 18 *(right panel)* two different hybridization bands were present. The two human papillomavirus type 18 hybridization bands in the cervicovaginal cells (lane *5*) represent the presence of a type 18 human papillomavirus with two Bam HI restriction sites and are characteristic of many human papillomavirus type 18 DNA samples identified to date (Burk RD, unpublished observations). Thus Patient D. B. had human papillomavirus 11 in the biopsy specimen (lane *4*) and both type 11 and type 18 in the cervicovaginal cells (lane *5*). The presence of human papillomavirus type 18 DNA in the cervicovaginal cells (lane *5*) probably resulted from a lesion in the cervix that was different from the biopsy specimen assayed (lane *4*).

Table II. Presence of human papillomavirus DNA in exfoliated cervicovaginal cells and findings of dysplasia in cervical biopsy specimens and Papanicolaou smears

Histologic results of biopsy specimen	*Papanicolaou smear*	*No. of patients with HPV DNA in cervicovaginal cells*
No abnormality	Class I	1/8*
	Class II	0/1*
	Class III/IV	1/1†
Dysplasia	Class I	6/6†
	Class II	3/3†
	Class III/IV	9/10†

HPV = Human papillomavirus; Class I = negative; Class II = atypical; Class III/IV = dysplasia/carcinoma in situ.

*Negative biopsy specimen with Class I or II Papanicolaou smear, one of nine (11%) with human papillomavirus present.

†Dysplasia and/or Class III/IV Papanicolaou smear, 19 of 20 (95%) with human papillomavirus present.

To determine whether the finding of human papillomavirus DNA in cervicovaginal cells was indicative of a dysplastic cervical lesion, we compared human papillomavirus DNA results obtained from cervicovaginal cells to the morphologic findings on Papanicolaou smears and cervical biopsy specimens in the 29 patients undergoing simultaneous colposcopy, Papanicolaou

Table III. Presence of human papillomavirus DNA in exfoliated cervicovaginal cells and findings on simultaneously obtained Papanicolaou smears

HPV DNA in cervicovaginal cells	*Papanicolaou smear*					
	Class I (negative)		*Class II (atypical)*		*Class III/IV (dysplasia/carcinoma in situ)*	
	n	%	*n*	%	*n*	%
Positive	10	29	5	45	16	94
Negative	24	71	6	55	1	6
Total	34		11		17	

smear, lavage, and biopsy (Table II). There was a high correlation between the finding of dysplasia on colposcopically directed biopsy and the presence of human papillomavirus DNA in cervicovaginal cells. Of 20 patients with dysplasia found on either biopsy specimen or Papanicolaou smear, 19 (95%) had human papillomavirus DNA detected in the cervicovaginal cells. In contrast, one of nine (11%) patients with no evidence of dysplasia in the biopsy specimen or Papanicolaou smear had human papillomavirus DNA in the cervicovaginal cells. The sensitivity of human papillomavirus DNA analysis of cervicovaginal cells as a screening test is corroborated by the finding that of the 20 patients with human papillomavirus DNA identified in cervicovaginal cells, 19 (95%) were shown to have a dysplastic lesion by standard histologic and cytologic methods. In contrast, 13 of 19 (68%) patients with dysplasia found in biopsy specimens had positive (Class II to IV) Papanicolaou smears on the day of study.

The results of human papillomavirus DNA hybridization in cervicovaginal cells were then compared to Papanicolaou smear results in a larger group of patients, some of whom had not undergone cervical biopsy analysis (Table III). Fifty-eight patients had one and two patients had two Papanicolaou smear and cervicovaginal cell specimens analyzed. Twenty-eight of 60 (47%) women had abnormal Papanicolaou smears and 32 of 60 (53%) had negative Papanicolaou smears on the day of study (Table III). In the 11 patients with Class II Papanicolaou smears, five (45%) had human papillomavirus DNA in the cervicovaginal cells. Of the 17 patients with Class III or Class IV smears, 16 (94%) had positive human papillomavirus DNA results.

Nine women with Class I (negative) smears (28%) had detectable human papillomavirus DNA in the samples of cervicovaginal cells. One patient had two smears taken 4 months apart; both were Class I and both lavage specimens contained human papillomavirus DNA. Smears from all 10 of these women were examined by a second cytopathologist, who confirmed that the samples were adequate and had no detectable abnormal cells.

To investigate the significance of the above findings (Table III), we examined the human papillomavirus DNA results and histopathologic findings in parallel biopsy specimens that were available in each group of patients with cervicovaginal cells either positive or negative for human papillomavirus DNA with negative Papanicolaou smears. None of the nine biopsy specimens from patients with negative cytologic results and negative cervicovaginal cell human papillomavirus DNA were positive for human papillomavirus DNA, whereas five of seven (71%) of the cervical biopsy specimens from patients with negative cytologic results and positive human papillomavirus DNA in the cervicovaginal cells were positive for human papillomavirus DNA. Moreover, histopathologic examination of biopsy specimens obtained simultaneously from patients with human papillomavirus DNA in the cervicovaginal cells but negative Papanicolaou smears demonstrated dysplasia in six of seven (86%). A retrospective review of the Papanicolaou smear results within a 1-year period revealed that six of nine (67%) patients who had negative cytologic results but positive human papillomavirus DNA in the cervicovaginal cells had had a Class III or IV smear in previous or subsequent specimens, whereas only four of 24 (17%) patients who had negative cytologic results and cervicovaginal cells negative for human papillomavirus DNA had had a Class III or IV smear in previous or subsequent specimens. Thus these negative Papanicolaou smears may represent faulty sampling (false negative) in at least 67% of the nine patients positive for human papillomavirus DNA in the cervicovaginal cells but with negative cytologic results.

Typing of human papillomaviruses in the 30 patients with human papillomaviruses DNA in the cervicovaginal cells was determined by restriction enzyme pattern and strength of hybridization with radiolabeled DNA probes from human papillomavirus types 6, 11, 16, and 18, and the results are shown in Table IV. At least five different types of human papillomaviruses were identified. Those samples that had neither strongest hybridization to a single human papillomavirus type nor characteristic restriction patterns were considered dis-

Table IV. Human papillomavirus types in exfoliated cervicovaginal cells correlated with Papanicolaou smear results

	Papanicolaou smear			
HPV DNA Type	*Class I*	*Class II*	*Class III*	*Class IV*
Negative	24	6	1	0
6	0	1	1	0
11	2	1	1*	1
16	6†	0	6	1
18	1	2	4*	0
"Other"	1	1	3	0

*One patient had both human papillomavirus type 11 and type 18 in cervicovaginal cells.
†One patient with human papillomavirus type 16 had two negative smears.

tinct from human papillomavirus types 6, 11, 16, and 18. In the 30 patients positive for viral DNA, human papillomavirus type 16 was detected in cervicovaginal cells from 12 (40%) patients, human papillomavirus type 18 in seven (23%), human papillomavirus type 11 in five (17%), human papillomavirus type 6 in two (7%), and "other" human papillomavirus types in five (17%). As illustrated previously (Fig. 1), one patient had both human papillomavirus type 11 and type 18 in the cervicovaginal cells. Human papillomavirus types 16, 18, and "other" were present in 14 of 17 (82%) women with Class III or Class IV Papanicolaou smears.

Comment

In this prospective study, we have collected exfoliated cervicovaginal cells by a painless, noninvasive cervicovaginal lavage technique in a group of women seen in a colposcopy clinic. We have detected human papillomavirus DNA in the exfoliated cells in 50% of such patients by restriction enzyme analysis and molecular hybridization. Since there are no other established methods to diagnose human papillomavirus infection, we have compared the analysis of human papillomavirus DNA in the lavaged samples with human papillomavirus DNA results in simultaneously performed colposcopically directed cervical biopsies. Detection of human papillomavirus DNA in cervicovaginal cells was correlated with the finding of human papillomavirus DNA in cervical biopsy samples, and, in fact, the human papillomavirus DNA was positive in the cervicovaginal cells even in cases in which the biopsy sample was negative. This probably results from the fact that the lavage technique samples exfoliated epithelial cells from the entire cervix, whereas the biopsy represents a focal sample. The detection of human papillomavirus DNA in cervicovaginal cells obtained by cervicovaginal lavage was indicative of a dysplastic cervical lesion in at least 95% of patients who had a colposcopically directed biopsy.

There is accumulating evidence from a variety of laboratories that human papillomavirus infection of the cervix may be one of the most important factors in the development of cervical dysplasia and carcinoma. However, the current standard method of cervical cancer screening is the Papanicolaou smear. Since the rate of false negative results obtained with analysis of a single Papanicolaou smear has been estimated to be between 10% and 50%,[10] the availability of another, more sensitive screening method is desirable to detect premalignant lesions of the cervix. Two recent reports identifying human papillomavirus infection in exfoliated cells from cervical swabs have identified human papillomavirus DNA by in situ filter hybridization (dot blot) in 83% (29 of 35) and 69% (49 of 71) of women with Class III or IV Papanicolaou smears.[24,25] In the present study, 94% (16 of 17) of patients with Class III or IV Papanicolaou smears had human papillomavirus DNA detected in cervicovaginal collected by lavage. It is noteworthy that the rate of detection of human papillomavirus DNA by the in situ filter hybridization method[16,17] in patients with Class I Papanicolaou smears was 11% (four of 36) and 2% (five of 229). In reviewing our experience, if the presence of human papillomavirus DNA is taken as an indication of a cervical lesion, the "false negative" cervical cytology rate for a single Class I Papanicolaou smear was 29%. In addition, those women with a Class I Papanicolaou smear and positive human papillomavirus DNA in the cervicovaginal cells had a very high incidence of another abnormal (Class III or IV) Papanicolaou smear in a 1-year time period and often had dysplasia on biopsy. The rate reported here of detection of human papillomavirus DNA in women with a single negative Papanicolaou smear is comparable to the rate of negative Papanicolaou smears obtained in women with an established dysplastic lesion either by the swab technique (51%) or by the swab-spatula technique (29%).[18]

In our study, the typing of different human papillomaviruses by restriction analysis and hybridization identified types 16 and 18 in 62% of patients with Class III and IV papillomavirus smears. In other studies, 65% (19 of 35) and 48% (27 of 56) had either human

papillomavirus type 16 and/or 18.[16, 17] Unfortunately, the use of dot blots as reported did not allow accurate typing of human papillomavirus DNA since the sample quantity may be limited and the identification is based on the strength of hybridization signal alone.[16, 17] An advantage of the lavage method reported here is the abundant quantity of exfoliated epithelial cells collected, which allows use of restriction analysis and separate hybridizations with multiple human papillomavirus probes to distinguish individual human papillomavirus types. By this method, it was clear that 17% (five of 30) of patients had a human papillomavirus type that was different from types 6, 11, 16, and 18. We detected one mixed infection (3%) with human papillomavirus types 11 and 18 (Fig. 1). Other groups have reported mixed infection in 7% (two of 29) and 14% (10 of 71) of patients.[16, 17] Since the biologic behavior and natural history are unknown for individual types of human papillomaviruses, we believe it is important to identify and study the clinical course of infection with each human papillomavirus type separately.

The results of the present study support the hypothesis that human papillomavirus infection of the cervix and associated dysplastic epithelial lesions may be part of the natural history of cervical cancer. The clinical significance of lavage screening with the finding of a specific type of human papillomavirus DNA in exfoliated cervical cells is the opportunity to identify associated dysplastic lesions by colposcopy that are amenable to therapy. A coordinated investigation of these findings is being undertaken to elucidate the propensity of lesions containing specific human papillomavirus types to progress. Although human papillomavirus types 16 and 18 are known to be present in the majority of cervical cancers,[2, 3] it is not known at present what percentage of cervical infections with these viruses will progress to malignancy. Moreover, the presence of acute versus persistent viral infection may significantly alter the risk of developing cervical cancer, similar to the observation that persistent infection with hepatitis B virus, and not simply a limited previous acute infection, is the predominant risk factor in developing hepatocellular carcinoma.[19] What is clear from our study and those of others is that human papillomavirus infection of the cervix is commonly found in women with abnormal Papanicolaou smears and in women who have dysplastic lesions, even in the presence of normal Papanicolaou smears. Although it is not known what the prevalence rate of human papillomavirus infection detected by DNA hybridization may be in a random screening of defined population groups, our data suggest a very high correlation between the findings of human papillomavirus DNA in cervicovaginal cells and an associated dysplastic cervical lesion. The detection of human papillomavirus DNA by the methods described here offers an objective assessment of human papillomavirus infection and can serve to identify those women who should be carefully evaluated for a dysplastic lesion of the cervix. The advantages of the method described here over the Papanicolaou smear as a screening test for precancerous lesions include: (1) greater sensitivity of detection of dysplastic lesions and (2) potential for greater prognostic information from human papillomavirus type than from morphologic results alone. Thus collection of exfoliated cells for human papillomavirus DNA detection by the lavage method offers a simple, noninvasive technique that can be performed in the gynecologist's office in conjunction with a molecular biology laboratory. Its usefulness as a generalized screening method and as a means of longitudinal evaluation is currently under study.

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Toxic shock syndrome, contraceptive methods, and vaginitis

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To elucidate further the etiology of toxic shock syndrome, we assessed the effects of certain contraceptive methods and recent history of vaginal infection on the incidence of toxic shock syndrome, with confounding effects of other risk factors controlled. We found a strong but imprecise positive association between toxic shock syndrome and tubal ligation (rate ratio = 7.9, 90% confidence interval 1.4 to 42.7). We also observed a negative association with oral contraceptives (rate ratio = 0.49, 90% confidence interval 0.22 to 1.1) and a positive association with a recent history of vaginitis (rate ratio = 2.1, 90% confidence interval 1.2 to 3.9). (Am J Obstet Gynecol 1986;154:989-91.)

Key words: Toxic shock syndrome, contraception, vaginitis

Tampon use in general and certain characteristics of tampons, such as brand and absorbency, have been reported to be important determinants of the incidence of toxic shock syndrome.[1] For the many women who continue to use tampons, however, little is known about preventive measures for reducing the risk of toxic shock syndrome. An apparent reduction in the incidence of toxic shock syndrome among users of oral contraceptives was observed[2] in the crude data from two early studies.[3, 4] The same inverse association is present in the crude data from two subsequent studies,[5, 6] as is a positive association with a recent history of vaginitis. The purpose of this analysis is to evaluate further the possible effects of specific contraceptive methods and vaginitis on the incidence of toxic shock syndrome by controlling the confounding effects of recognized risk factors.

Methods

We obtained a computer tape of data from the Tri-State study,[6] the largest study of toxic shock syndrome conducted to date. To verify the data, we reproduced the published results.[6] The Tri-State study included all 76 nonfatal cases of toxic shock syndrome occurring in women during a menstrual period that were reported to the Iowa, Minnesota, and Wisconsin state health departments between October 1, 1979, and September 19, 1980. For each case, two controls were selected and matched to the case by date of birth and neighborhood of residence. All controls were required to have experienced a menstrual period within 2 months of onset of toxic shock syndrome in the matched case. Other details of the design and conduct of the study have been published previously.[6]

The present analysis was restricted to the 75 cases and 123 controls who were menstruating and using tampons at the time of toxic shock syndrome onset. Information about menstruation, tampons, and contraceptive methods relates to the time of the index menstrual period, which is defined for cases as the menstrual period during which toxic shock syndrome occurred. For controls, it is defined as the menstrual period closest in time to the index menstrual period of the matched case. We estimated effects of seven contraceptive methods reported by the women in this study: condom, diaphragm, foam, intrauterine contraceptive device, oral contraceptives, tubal ligation, and vasectomy. The unexposed group comprised women

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Table I. Exposure frequencies and RR estimates of toxic shock syndrome among tampon users by method of contraception and history of vaginitis

	Exposure frequency				
	Cases (n = 75)	*Controls (n = 123)*	*Crude RR*	*Standardized RR*	*90% Confidence interval*
Contraceptive method					
Condom	3	1	4.7	2.5	0.39-15.9
Diaphragm	3	5	0.95	0.95	0.24-3.4*
Foam	0	3	0	0	0-1.9*
Intrauterine contraceptive device	3	3	1.6	1.6	0.36-6.9*
Oral contraceptives	10	27	0.59	0.49	0.22-1.1
Tubal ligation	3	1	4.7	7.9	1.4-42.7
Vasectomy	3	4	1.2	1.2	0.29-4.6*
Vaginitis	21	19	2.1	2.1	1.2-3.9*

*Confidence interval reported for crude estimate.

who reported using no contraception (50 cases, 79 controls). For the analysis of vaginitis, comparison was made to women reporting no history of vaginitis in the past year (54 cases, 104 controls).

A summary rate ratio (RR) estimate for each contraceptive method and for vaginitis across categories of potentially confounding variables was obtained by standardizing the RR according to the distribution of each confounding variable among the controls in the high-risk stratum of the risk factor being evaluated.[7] To control for the matching variables, uniform RR estimates were computed by the method of maximum likelihood.[8] We considered as potentially confounding variables age, neighborhood, tampon brand (Rely brand/other brands), tampon fluid capacity, vaginitis (for analysis of contraceptive methods), and the specific contraceptive methods (for analysis of vaginitis). A comparison of the standardized RR to the crude (unstandardized) RR indicates the degree of bias attributable to the confounding variable.[7] When adjustment was indicated we reported the standardized estimate; otherwise we reported the crude estimate of RR. Approximate confidence intervals for the standardized RR were calculated according to a Taylor series approximation, and for crude estimates of RR we calculated exact confidence intervals.[9] We evaluated the degree of random error in the estimates of RR by examining the widths of the confidence intervals.[10]

Results

Estimates of RR for each type of contraceptive method and vaginitis are presented in Table I. Tubal ligation is associated with a marked increase in the incidence of toxic shock syndrome (RR = 7.9). The confidence interval is wide, indicating that the estimate of RR is imprecise, but the position of the interval clearly denotes a positive association that is likely to be strong.

The data show a negative association for oral contraceptive use (RR = 0.49). The confidence limits indicate that, with respect to random error, these data provide moderately strong evidence of a reduction in the incidence of toxic shock syndrome among oral contraceptive users.

A history of vaginitis in the past year is associated with a doubling of the incidence rate of toxic shock syndrome (RR = 2.1). The confidence limits indicate that the estimate is sufficiently precise to provide considerable evidence of a positive association.

The estimates of RR for use of condom, diaphragm, foam, intrauterine contraceptive device, and vasectomy are very imprecise, and therefore the data provide only scant information about the relation of these contraceptive methods to the incidence of toxic shock syndrome.

Comment

The strong association between toxic shock syndrome and tubal ligation has not been reported previously. The association is based on only three exposed cases, so the explanation for this result is unclear; however, a causal interpretation would have important implications for the increasing number of young women choosing this method of contraception.[11] Of the cases reporting a tubal ligation, all reported having had the procedure at least 1 year before the onset of toxic shock syndrome, thereby precluding an acute effect of the operation. Previous analyses have combined tubal ligation with other contraceptive methods, thus preventing a comparison with the results we report.

A negative association between oral contraceptive use and toxic shock syndrome has been reported.[2] Our analysis demonstrates that the apparent reduction in the toxic shock syndrome incidence rate among oral contraceptive users cannot be explained by confounding effects of recognized risk factors. A protective effect of oral contraceptives against pelvic inflammatory dis-

ease has been suggested,[12] and it is possible that oral contraceptives protect against several kinds of vaginal infection.[2]

An approximate doubling of the incidence rate of toxic shock syndrome is observed for women reporting a history of vaginitis in the previous year. Crude data from one previous study show a weak positive association for this exposure (RR = 1.4).[5] Recurrent toxic shock syndrome has been reported among menstruating women[3]; women reporting a history of vaginitis in the past year may have experienced prediagnostic symptoms of toxic shock syndrome. An alternative explanation is that toxic shock syndrome shares etiologic factors with other forms of vaginal infection.

The range of associations we report for various types of contraceptive methods and for vaginitis makes it difficult to attribute these results to a common bias. The association for oral contraceptives is in the opposite direction from the association for tubal ligation. In order for both of these results to be explained by bias, at least two sources of bias would have to be proposed. Each would have to be specific to the particular method and unrelated to contraceptive use or to sexual activity in general. Yet another explanation would be required for the vaginitis association seen in these data.

This analysis provides evidence about possible risk factors for toxic shock syndrome that have received little attention thus far. Although more evidence is needed before the relations reported here can be clearly understood, the results suggest that the etiology of toxic shock syndrome may involve a variety of gynecologic factors in addition to menstruation and tampon use.

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Necessity of endocervical curettage in colposcopy

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The use of endocervical curettage was evaluated in 200 patients undergoing colposcopy, biopsy, endocervical curettage, and conization. It is suggested that endocervical curettage be performed only in cases of satisfactory colposcopy, since patients with unsatisfactory colposcopy should undergo conization. (AM J OBSTET GYNECOL 1986;154:992-5.)

Key words: Endocervical curettage, colposcopy, biopsy, conization

With the use of colposcopy to evaluate cervical lesions, the management of women with abnormal Papanicolaou smears has become more individualized, with fewer indications for diagnostic conization. To improve the diagnostic accuracy before outpatient treatment of cervical intraepithelial neoplasia, Townsend and Richart[1] have suggested that endocervical curettage be a standard part of a colposcopic examination. This suggestion has not been accepted by all authors.[2-7]

Our present study is undertaken to determine the utility of endocervical curettage in evaluating patients who have both satisfactory and unsatisfactory colposcopic examinations.

Material and methods

Between 1981 and 1983, a total of 824 patients were seen in the dysplasia clinic of the University of Texas Medical Branch at Galveston; 263 of these patients (32%) underwent conization of the cervix. Data concerning cervical biopsies, endocervical curettage, and conization of the first 200 patients were included in this study. No patient was pregnant at the time of evaluation. Cervical dysplasia was classified according to Richart's scheme[8]: grade 1, 2, or 3 cervical intraepithelial neoplasia. Microinvasion was defined as stromal invasion of 3 mm or less below the basement membrane of the epithelium from which it arose, without vascular or lymphatic involvement or confluence of neoplastic tongues. When more than one specimen was collected, the most severe diagnosis was reported. Cervical biopsies and cone specimens were categorized according to the degree of dysplasia. The findings of endocervical curettage were classified as positive if any dysplastic cells were noted on the curettage specimen.

Results

Of 200 patients undergoing conization, 113 (57%) had unsatisfactory and 87 (43%) had satisfactory colposcopic examinations.

The degree of cervical intraepithelial neoplasia on cervical biopsy specimens was compared to the positive or negative results of the endocervical curettage. Among 113 patients with unsatisfactory colposcopy (Table I), 62 (55%) had a negative finding on endocervical curettage and 51 (45%) had a positive finding. Three patients (3%) with negative biopsy results had a positive finding on endocervical curettage. Among 87 patients with satisfactory colposcopy who were undergoing conization for other reasons (positive endocervical curettage specimen, wide geographic disease, Papanicolaou smear, or biopsy suggestive of invasion) (Table II), 18 (21%) had a negative endocervical curettage finding and 69 (79%) had a positive finding. Eleven patients (13%) who had negative biopsy results had a positive endocervical curettage finding.

The degree of cervical intraepithelial neoplasia on conization specimens was compared to the positivity and negativity of the endocervical curettage specimens. Among 62 patients with an unsatisfactory colposcopy and a negative endocervical curettage finding (Table III), 50 (81%) had a dysplasia of the cervix on conization specimens ranging from grade 1 to grade 3 cervical intraepithelial neoplasia. None had invasive carcinoma. Among 51 patients with an unsatisfactory colposcopy and a positive endocervical curettage specimen (Table III), 49 (96%) had dysplasia of the cervix on conization specimens ranging from grade 1 cervical intraepithelial neoplasia to invasive carcinoma. It is to be noted that 30 patients had grade 3 cervical intraepithelial neoplasia and six had microinvasive disease. Among 18 patients with a satisfactory colposcopy and a negative en-

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Table I. Comparison of degree of cervical intraepithelial neoplasia on cervical biopsy specimens versus positive or negative endocervical curettage results in patients with unsatisfactory colposcopy (N = 113)

	Cervical biopsy diagnosis					
		Cervical intraepithelial neoplasia				
Endocervical curettage diagnosis	*Negative*	*Grade 1*	*Grade 2*	*Grade 3*	*Invasive carcinoma*	*Total*
Negative	13	6	16	27	0	62
Positive	3	8	10	28	2	51
Total						113

Table II. Comparison of the degree of cervical intraepithelial neoplasia on cervical biopsy specimens versus positive or negative endocervical curettage results in patients with satisfactory colposcopy (N = 87)

	Cervical biopsy diagnosis					
		Cervical intraepithelial neoplasia				
Endocervical curettage diagnosis	*Negative*	*Grade 1*	*Grade 2*	*Grade 3*	*Invasive carcinoma*	*Total*
Negative	1	2	4	10	1	18
Positive	11	11	19	28	0	69
Total						87

Table III. Comparison of the degree of cervical intraepithelial neoplasia on cone specimens versus positive or negative endocervical curettage in patients with unsatisfactory colposcopy (N = 113)

	Final cone diagnosis					
		Cervical intraepithelial neoplasia			*Microinvasive or invasive cancer*	
Endocervical curettage diagnosis	*Negative*	*Grade 1*	*Grade 2*	*Grade 3*		*Total*
Negative	12	3	23	24	0	62
Positive	2	4	9	30	6	51
Total						113

docervical curettage finding (Table IV), 18 (100%) had dysplasia of the cervix on conization specimens ranging from grade 1 cervical intraepithelial neoplasia to invasive carcinoma. Among 69 patients with satisfactory colposcopy and positive endocervical curettage results (Table IV), the final diagnosis on conization specimens was grade 1 cervical intraepithelial neoplasia to invasive disease in 59 patients (85%). One patient had microinvasion and one had invasive disease.

Ten cases of microinvasive carcinoma and one case of invasive carcinoma were diagnosed among 200 patients undergoing conization (5.5%). It is of interest that the endocervical curettage had negative results for dysplasia and that the colposcopy was satisfactory in three cases. The reasons for conization were a wide geographic lesion associated with a biopsy revealing grade 3 cervical intraepithelial neoplasia in two cases and a cervical biopsy suggesting invasive disease in one case.

Comment

The endocervical curettage, which was a required step in this study, has been advocated by many authors. Townsend et al.[9, 10] recommended endocervical curettage with all colposcopic examinations and subsequent conization in the presence of dysplastic disease in the canal. In studying patients with invasive carcinoma missed after colposcopic examination, they stated that the most common omissions were the failure to perform an endocervical curettage and the failure to take an adequate number of biopsy specimens. Shingleton et al.[11] used endocervical curettage in 603 patients. They emphasized that curettings are more reliable than endocervical smears for the diagnosis of cervical neoplasia and proceeded to definitive therapy, either cryotherapy or hysterectomy, without prior conization if the curettings were no worse than moderately dysplastic. They have found that the cervical biopsy diagnosis was the same as the final pathologic diagnosis in 86% of cases with negative endocervical curettage findings but in only 68% of cases with positive endocervical curettage findings. Kelley et al.[12] reviewed 250 patients who had conization or hysterectomy following colposcopic biopsies and endocervical curettage. Biopsy and endocervical curettage diagnoses corresponded

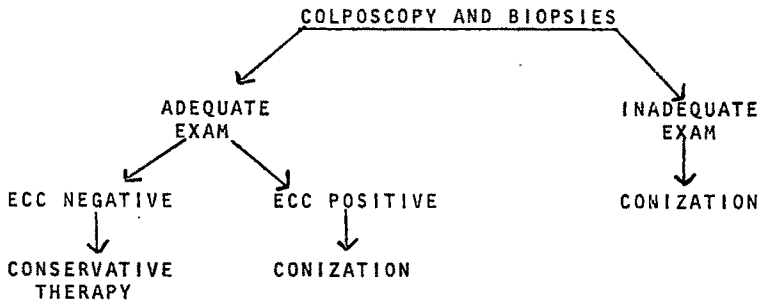


Fig. 1. Evaluation of abnormal Papanicolaou smears at The University of Texas Medical Branch at Galveston.

Table IV. Comparison of the degree of cervical intraepithelial neoplasia on cone specimens versus positive or negative endocervical curettage results in patients with satisfactory colposcopy (N = 87)

	Final cone diagnosis					
		Cervical intraepithelial neoplasia			*Microinvasive or invasive cancer*	
Endocervical curettage diagnosis	*Negative*	*Grade 1*	*Grade 2*	*Grade 3*		*Total*
Negative	0	2	4	9	3	18
Positive	10	5	19	33	2*	69
Total						87

*One invasive carcinoma.

with the final diagnosis in 92% of cases. No invasive cancer was missed. Hatch et al.[13] also advocated the use of endocervical curettage in all cases. In 125 patients with inadequate colposcopy, nine patients had invasive cancer and five patients, microinvasive disease. All of these cases were associated with a positive finding on endocervical curettage. The authors concluded that the endocervical curettage gave valuable information for outpatient treatment and eliminated the need for conization in 76% of patients after unsatisfactory examinations.

Conversely, other authors do not perform an endocervical curettage, or they use it only in cases of unsatisfactory colposcopy. Talebian et al.,[2] using only colposcopically directed biopsies, found 100% accuracy in cases of satisfactory colposcopy in 114 patients undergoing conization or hysterectomy. On the contrary, among 54 patients with unsatisfactory colposcopic examination, 56% showed more advanced disease on conization. Swan[3] used colposcopically guided biopsies without endocervical curettage in 763 patients and reported a high degree of accuracy (98.4%) comparable to other reports. Javaheri and Fejgin,[4] in a series of 930 patients, concluded that omission of endocervical curettage in patients with satisfactory colposcopy did not reduce the accuracy of the diagnosis. He advised, however, the performance of an endocervical curettage in all patients with unsatisfactory colposcopy. After adequate colposcopy an exact diagnosis was reached in 76.5% of cases; after inadequate colposcopy a more advanced lesion was detected in over 50% of cases. Urcuyo et al.[5] noted that among patients with adequate colposcopy, 8.6% had positive endocervical curettings, but a more severe lesion was never found in comparison with cervical biopsies, conization, or hysterectomy. In patients with unsatisfactory colposcopy, 57.3% had positive endocervical curettings, and in two patients the curettings showed invasive cancer. He concluded that endocervical curettage was of little value in cases of adequate colposcopy results but was indicated when results were unsatisfactory, since a frank invasive carcinoma may be diagnosed and conization avoided.

On the other hand, Rochelson and Krumholz[6] withheld endocervical curettage in patients with unsatisfactory colposcopic examinations because it increases patient discomfort and because the patients were to undergo conization subsequently. Drescher et al.,[7] in a review of 540 patients, reported positive endocervical curettage findings in 17.9% of patients with satisfactory colposcopy and in 48.7% of patients with unsatisfactory colposcopy. In cases of both satisfactory and unsatisfactory examinations, the diagnosis on endocervical curettage was more significant than that with the cervical biopsies in 3.8% and 21.1% of cases, respectively. They advocated that endocervical curettage be done in all patients except those for whom cone biopsy is planned.

Our experience seems to agree with the findings of Rochelson and Krumholz[6] and of Drescher et al.[7] Positive endocervical curettage results were the most frequent reason (58%) for conization in our patient pop-

ulation. The endocervical curettage results were positive in 45% (51 of 113) of patients with unsatisfactory colposcopy and 10.6% (69 of 648) of patients with satisfactory colposcopy.

The data from this study can be used to answer the question, does endocervical curettage add anything to an adequate colposcopic examination with colposcopically directed biopsies? The data support an affirmative answer because 79% of patients had positive results for dysplasia on endocervical curettage. Among those patients, 13% had normal cervical biopsies (Table II). In addition, 85% of patients undergoing conization had dysplasia of the cervix ranging from grade 1 cervical intraepithelial neoplasia to invasive disease. One case of invasive carcinoma and one case of microinvasive carcinoma would have been missed had the endocervical curettage and subsequent conization not been performed (Table IV). The positivity of endocervical curettage findings is one of the reasons for conization in these patients with satisfactory colposcopic examinations.

The second question concerns the utility of endocervical curettage after an unsatisfactory colposcopic examination. Although 45% of patients had positive results for dysplasia on endocervical curettage, among which two cases showed possible invasion by biopsy (Table I), those patients would not have avoided conization because of the unsatisfactory examinations. After unsatisfactory colposcopy, an endocervical curettage adds nothing to the diagnosis. Even the diagnosis of frank invasive disease cannot be made affirmatively on endocervical curettings because of the scant stroma. Furthermore, a previous endocervical curettage hampers the pathologic interpretation of the cone specimen if conization is performed soon after endocervical curettage. For these reasons, we believe the endocervical curettage can be omitted in all cases after unsatisfactory colposcopy, since conization is indicated (Fig. 1).

In all cases, however, correlation between cytologic findings, colposcopic findings, biopsy results, and endocervical curettage results should be strictly maintained before outpatient treatment is given or surgery is performed.[14]

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Physiologic changes in pregnant women and their fetuses during jet air travel

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The physiologic reactions of pregnant women and their fetuses were studied during routine commercial flights. Ten healthy pregnant women (32 to 38 weeks of gestation) each undertook two flights. Maternal respiratory and heart rates, transcutaneous Po_2 and Pco_2, blood pressure, uterine activity, and fetal beat-to-beat heart rate variability were continuously monitored. During these flights, maternal heart rate and blood pressure increased, and Po_2 decreased significantly while Pco_2 remained unchanged. Respiratory rate showed a short increase during takeoff and landing but remained unchanged during the rest of the flight. Mean fetal heart rate was within normal limits during the whole flight. No bradycardia, prolonged tachycardia, or significant loss of heart rate variability was observed. This study indicates no hazards of commercial flights to the mother and the fetus in uncomplicated pregnancies. (Am J Obstet Gynecol 1986;154:996-1000.)

Key words: Pregnancy, fetus, altitude, hypoxia, maternal anxiety, jet air travel

Obstetricians vary in their advice to pregnant women regarding air travel. These divergent opinions result partly from lack of knowledge of the physiologic adaptations of the maternal-fetal unit during flight. There are at least two significant questions that require investigation: Can the emotional stress of the mother during flight lead to reduced uterine circulation? What is the effect on the mother and the fetus of short-term altitude exposure?

Modern aircraft maintain a cabin pressure corresponding to an altitude between that of the airport and 2500 m (8200 feet). Consequently, the oxygen partial pressure in the cabin air is reduced. Both theoretical calculations and measurements in healthy adults give arterial Po_2 values between 60 and 70 mm Hg under the conditions occurring during commercial flights.[1] The effect on the fetus of this reduction of maternal Po_2 is unknown. Theoretical discussions of this question by Cameron,[2] Parer,[3] and Scholten[4] have concluded that given normal placental function, the fetus should be unaffected. However, there are no studies under simulated or actual flying conditions to support this conclusion. To help clarify the effect of air travel on pregnant women and their fetuses, we studied mothers and fetuses during commercial flights.

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Material and methods

The study was performed on Swissair flights between Zurich, Switzerland, and Amsterdam, The Netherlands, or Zurich, Switzerland, and Copenhagen, Denmark. The aircraft used was a DC 9 and the flight time was 1 to 1½ hours. Measurements were repeated on each mother during the return flight. Approval for this study was obtained from the Ethical Committee of the University. Table I gives details of the pregnant volunteers and the variables monitored.

Each flight was divided into five phases: takeoff, climbing, cruising, descent, and landing. As seen in Fig. 1, the data were analyzed with respect to the different phases of the flight. Within each phase mean values of 6-minute periods were calculated. The method of Fallenstein et al.[5] was used to analyze fetal heart rate variability. Paired *t* tests were used for statistical comparisons.

Results

No complications occurred during flight and subsequently all the women were delivered of healthy infants.

Flight data. The cabin pressure at maximal altitude averaged 586 (555 to 597) mm Hg corresponding to a simulated altitude of 2395 (2635 to 1900) m or 7855 (8642 to 6227) feet. This level of cabin pressure was reached at an average of 15 minutes after takeoff.

The mother

Transcutaneous blood gases. As expected, maternal transcutaneous Po_2 fell during flight (Fig. 1) and paralleled the decrease in cabin pressure. The mean fall in transcutaneous Po_2 at peak altitude was 25%.

Table I. Characteristics of the women studied and the variables measured

Pregnant volunteers	
Total number	10
Mean age (yr)	28
Primiparous	7
Smoker	2
Length of gestation (wk)	32-38
Parameter monitored *(continuously and noninvasively)*	Instrument used
Maternal transcutaneous P_{O_2}	Self-developed equipment
Maternal transcutaneous P_{CO_2}	
Maternal respiratory rate	Cardiorespirograph, Hellige, Freiburg, Federal Republic of Germany
Maternal heart rate (from electrocardiogram)	
Maternal blood pressure, systolic	Dinamap, Applied Medical Research, Tampa, Florida
Maternal blood pressure, diastolic	
Maternal uterine pressure (external)	Cardiotocograph HP 8040A, Hewlett-Packard, Böbligen, Federal Republic of Germany
Fetal heart rate (ultrasound Doppler)	
Cabin pressure (electronic barometer)	Self-developed equipment

No change, such as a fall due to hyperventilation, was seen in transcutaneous P_{CO_2}, which was constant in all patients throughout the flights.

Respiratory rates. Mean maternal respiratory rates were between 20 and 26 breaths per minute in the various flight phases. Before and during takeoff there was some increase in respiratory rate, but respiratory rate then decreased and remained stable in subsequent phases of the flight. In one woman ventilation briefly increased to a rate of 70 breaths per minute during landing of the airplane.

Heart rate. The results of the maternal heart rate recordings are seen in Fig. 1. Baseline heart rate was 86 bpm and increased gradually during the flight. In addition a peak was seen during takeoff and a second peak was reached at maximal altitude. This level was significantly higher than the initial peak during takeoff ($p < 0.001$). Another small rise was noted during landing. Individual increases up to 120 bpm were noted.

Five of the women showed cyclic changes in heart rate as illustrated in Fig. 2. They had an amplitude of about 20 bpm and appeared with intervals of 2 to 3 minutes. The return to the initial baseline heart rate was always associated with a contraction (see *arrows* in Fig. 2).

Blood pressure. Mean systolic and diastolic blood pressures, respectively, ranged between 116 and 124 mm Hg and between 66 and 74 mm Hg. The diastolic pressure fell during ascent ($p < 0.001$) but both values were

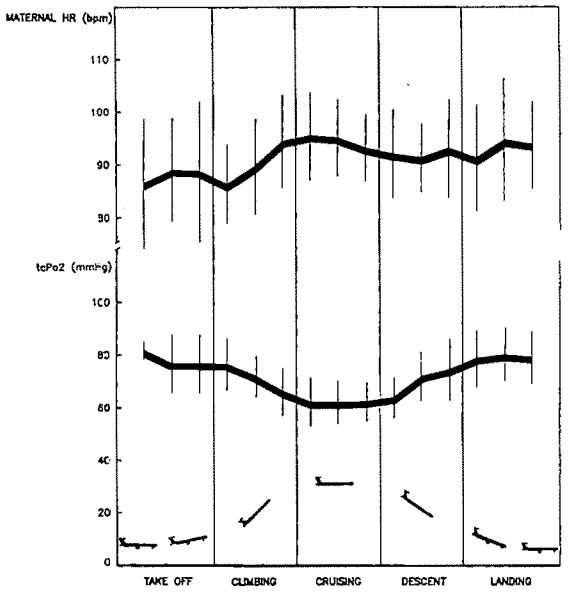


Fig. 1. Mean (±SD) maternal (10 women, 20 flights) heart rate and transcutaneous P_{O_2} *($tcPo_2$)* values during the various flight phases.

increased over baseline at peak altitude. Upon landing, blood pressure returned to preflight levels.

Uterine activity. The regular contractions discussed in connection with the cyclic heart rate changes were not felt by the mother. The frequency of sporadic contractions was consistent with the time of gestation.

The fetus. Fig. 3 illustrates the changes in the mean baseline fetal heart rate. There was a small but statistically significant ($p < 0.02$) increase during takeoff and during approach to landing ($p < 0.05$). In all flight phases, however, mean baseline fetal heart rate was within normal limits. The mean amplitude of the long-term variability of the fetal heart rates can be seen in the lower part of Fig. 3. There is no indication of reduced fetal heart rate variability.

Individual heart rate patterns in seven fetuses showed no significant change related to particular flight phases. Only three times during takeoff and ascent and once at cruising altitude (three fetuses) were marked accelerations (three of short duration and one of 8 minutes) noted.

Comment

Man cannot tolerate the low oxygen pressure where ordinary jet aircraft cruise, that is, 9000 to 12,000 m (30,000 to 40,000 feet) and 15,000 to 18,000 m (50,000 to 80,000 feet) for the Concorde, so all commercial jet airlines are pressurized. To reduce weight and cost, compression of incoming air is limited so that the resulting oxygen tension is equivalent to that at 1500 to 2450 m (about 5000 to 8000 feet). Many women live permanently at these altitudes, for example, in Switzer-

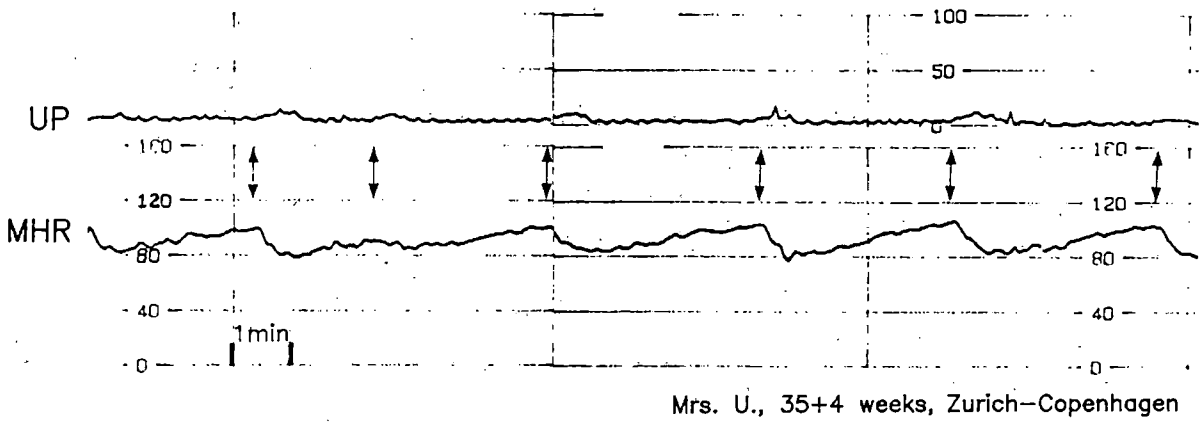


Fig. 2. Recordings of maternal heart rate *(MHR)* and external uterine pressure *(UP)* in a pregnant woman during flight showing the relationship between slight uterine contractions and maternal heart rate fluctuations *(arrows)*.

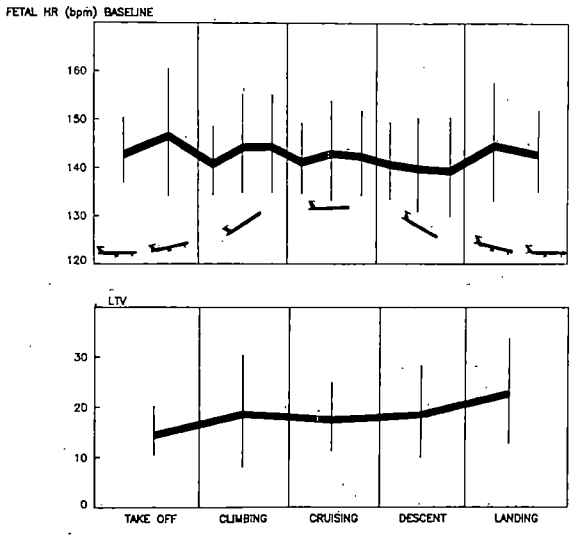


Fig. 3. Mean (±SD) fetal (10 fetuses, 20 flights) beat-to-beat heart rate baseline and long-term variability *(LTV)* in the various flight phases.

land or North and South America, and there is no evidence of any adverse consequence for pregnancy. However, at 3000 m and above, the evidence for a negative influence of the resulting hypoxia on reproduction is unanimous. Systematic studies (reviewed by Huch[6]) controlling for the multifactorial influences of race, low socioeconomic status, cold, and radiation have demonstrated the adverse role of reduced oxygen pressure in fetal outcome. However, there are several mechanisms of mother and fetus to adjust to life at high altitude but because of lack of time to adapt to short-term exposure to conditions in flight equivalent to altitudes of about 2500 m, some negative influence on the fetus might be anticipated even at these heights.

Under normal flight conditions, the lowest oxygen partial pressure to which passengers should be exposed is about 110 mm Hg. This corresponds to an oxygen concentration of 15% at sea level and represents a reduction of about 28% from normal. This agrees with our findings that maternal transcutaneous Po_2 fell by a mean of 25% from the initial value in Zurich (altitude 440 m). Wulf[7] studied pregnant women inhaling a gas mixture containing 15% oxygen. The mean arterial Po_2 value in 63 women was 65 mm Hg and similar results have been obtained in non-pregnant adults.[1, 8]

The decrease in Po_2 in our patients occurred without a significant change in respiratory rate. The small increases in respiratory rate noted at takeoff and landing were felt to be emotionally induced by these phases of the flight. No direct measurement of the tidal volume was performed, but the stable transcutaneous Pco_2 values coupled with a stable respiratory rate indicate no major change in tidal volume. The results clearly show that flying does not augment the already existing state of hyperventilation in pregnant women.[9, 10]

Pregnant women as well as nonpregnant adults[8, 11, 12] react to a reduction in inspired oxygen content with an increase in heart rate and systolic pressure. In our study the increase in maternal heart rate paralleled the drop in transcutaneous Po_2. Additional factors may also be involved as the maternal heart rate did not return completely to baseline at the end of the flight. It may for instance be relevant that the mothers and the equipment were observed and discussed by the other passengers.

Because of the instrumentation the mothers were partially immobilized in the sitting position during the flights. This positioning may explain the cyclic heart rate accelerations noted in five of the 10 women studied (Fig. 2). We previously described in late pregnancy a similar reaction with standing.[13] Pronounced phasic maternal heart rate accelerations occur in order to compensate for a reduced venous return from the lower

extremities. During standing venous return is impaired because of compression by the uterus, analogous to the vena cava syndrome in supine woman in late pregnancy. We have seen that during standing venous return improves phasically with uterine contractions. Such changes may also be associated to a lesser degree with sitting. Whether this reaction is detrimental to the fetus is presently not known, but it may be wise to encourage pregnant passengers to move about more often than they might otherwise do, especially on long-distance flights where one might anticipate an aggravation of any problem related to immobilization. Besides a lower oxygen tension there are other hazards that may be a function of flight duration, such as tobacco smoke, ozone, and cosmic rays. Whether these additional factors have a negative influence on mother and fetus during flight is not known. On the other hand, the effect of maternal hypoxemia that we have been discussing might be even less in transatlantic flights where cabin pressures tend to be higher than on the short-distance flights that we studied.

Fetal heart rate in flight was analyzed for evidence of fetal distress according to the usual antepartum or intrapartum criteria. In the present study the heart rate recordings obtained in all fetuses were of good technical quality and could be reliably assessed for the presence or absence of distress. The data do not reveal any convincing indications that the fetuses had to compensate for reduced blood flow secondary to maternal anxiety or maternal hypoxia induced by altitude. Notable fetal heart rate accelerations occurred only three times in the takeoff and initial climbing phases. As they were not paralleled by maternal heart rate accelerations, we believe that they reflect fetal arousal reactions resulting from vibration and accelerations during takeoff rather than changes secondary to maternal stress. With an exception of an 8-minute acceleration period to about 180 bpm in one fetus at cruising altitude who on the return flight did not show any heart rate abnormality, heart rate behavior of the fetuses was not suspicious for hypoxia at any instant during 30 hours of fetal monitoring during flight.

The results of our study during flight show moderate but significant cardiopulmonary changes in mothers but no significant fetal reactions. We are convinced that for normal uncomplicated pregnancies, flying as a passenger in modern aircraft can be recommended without restrictions. Late pregnancies were chosen for study because physiologic changes are most marked at this time and fetal monitoring is technically easier. As we are not aware of any data showing improved adaptation to stress and hypoxemia with increasing gestational age, we believe we can extrapolate our findings in late pregnancy to include the entire gestational period. However, an aircraft is certainly not a suitable place to give birth and it is obvious that airline restrictions on the use of air transportation during the final weeks of pregnancy should be observed whenever possible.

The situation of the pregnant stewardess may be different because here, in addition to the altitude exposure, the effect of physical work on mother and fetus needs to be considered.[14] Yoshioka et al.[15] studied nonpregnant flight attendants on the ground and during flights (cabin pressures corresponding to 1500 m (4920 feet) in DC 10 and 747 aircraft) and found their work to be quite strenuous. Heart rate increases for the same work were always more pronounced at altitude compared to those at ground level. Even simple walking in the aisle of the cabin at several degrees of inclination produced a significant rise in heart rate. Our analogous studies in the Swiss mountains (Laax, at 2200 m, 7200 feet) with pregnant volunteers who exercised for 3 minutes on a bicycle at 25 W resulted in maternal heart rate changes of a similar order of magnitude. With one exception, however, the fetuses did not display any signs of distress. This exception occurred in a pregnant woman who smoked and whose fetus exhibited a brief period of bradycardia after exercise. This stresses the need for careful examination and evaluation of additional risk factors that might impair fetal capacity to adjust to reduced uterine blood flow and maternal hypoxemia.

In summary, flying in modern commercial aircraft should not be considered harmful to the pregnant woman and her fetus if additional risk factors are excluded.

We acknowledge the cooperation of the 10 pregnant women without whom this study would not have been possible.

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Moderate recreational running is associated with lowered salivary progesterone profiles in women

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Salivary progesterone profiles were studied in a group of recreational women runners (average 12.5 miles per week) and nonexercising control subjects. Although no differences were observed in the average cycle lengths of the two groups, luteal progesterone levels, both peak and average, were found to be significantly lower in the runners. A salivary progesterone level two standard deviations above the follicular phase average was used to discriminate luteal activity with 95% confidence. Runners averaged fewer days with sample values two standard deviations above this level and a shorter average interval between first and last samples observed above this level. However, no evidence was found of a delayed luteal progesterone rise among the runners. These results suggest that even moderate amounts of aerobic exercise may have effects on female reproductive function which would not be suspected from menstrual patterns alone. (AM J OBSTET GYNECOL 1986;154:1000-3.)

Key words: Progesterone, luteal phase, exercise, menstrual cycle, saliva

Numerous recent reports have provided evidence of a suppression of reproductive function in women athletes. Studies of menstrual histories have shown an increased incidence of oligomenorrhea and amenorrhea among competitive runners[1, 2] and swimmers[3] and among ballet performers,[4] relative to age-matched controls. Studies of acute hormonal responses have shown that exercise leads to increased levels of circulating steroids in women, but not always of gonadotropins.[5, 6] In addition, elevated concentrations of hormones with antireproductive properties have been observed in women following exercise.[3, 6, 7]

Chronic hormonal responses to regular exercise are less well documented. Shangold et al.[8] report a significant suppression of luteal phase progesterone secretion in a single runner and a negative correlation in the same subject between length of the luteal phase (determined by cervical mucus changes) and weekly training mileage. It is noteworthy that no differences in overall cycle length were observed between control and training cycles in this subject. Bonen et al.[9] compared hormone profiles of four elite teenage swimmers for a single month with age-matched controls and fertile adults. Luteal progesterone was also lowered and luteal phase length shortened in this group compared to both control groups. Most recently Bullen et al.[10] reported that increased incidence of menstrual irregularities, low progesterone levels, and absent luteinizing hormone peaks could be induced by subjecting volunteers to a 2-month exercise regimen beginning with running 28 miles/week, progressing to 70 miles/week, and including 3½ hr/day of additional aerobic exercise.

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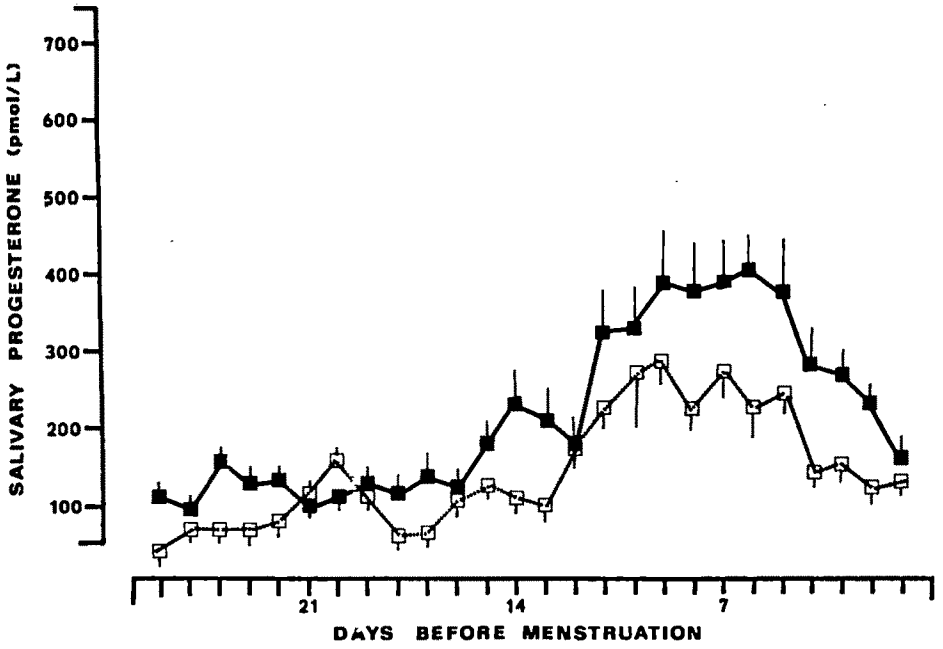


Fig. 1. Comparison of the composite salivary progesterone profiles of runners *(open squares)* and controls *(solid squares)*, aligned on day of menstrual onset. *Vertical lines* represent standard error of the group mean for that cycle day.

Although extreme levels of exercise thus often disrupt or shorten menstrual cycles, the study reported by Shangold et al.[8] suggests that milder regimens of exercise may lead to a suppression of luteal function that would not be observed from an examination of menstrual histories. Detection and quantification of mild luteal suppression require frequent monitoring of circulating progesterone levels. We have developed an assay of salivary progesterone to facilitate research in this area and used it to compare luteal progesterone levels in a group of recreational women runners with a group of nonathletic control subjects. In so doing we attempt to determine (1) whether luteal suppression is observable even among women following moderate exercise regimens and (2) how such suppression is characterized in terms of the timing, extent, and duration of progesterone secretion.

Material and methods

Subjects were recruited from an amateur athletic club in Boston and from the Harvard University community, although Harvard undergraduates were not included. Saliva samples from 15 cycles were collected from eight women identified as recreational runners averaging 12.5 ± 0.8 (mean ± standard error) miles of running exercise a week. Samples from 19 cycles were collected from a group of nine controls who reported no regular exercise habits. All subjects were screened for oral contraceptive use, recent pregnancy, and menstrual history. No attempt was made to control for marital status, socioeconomic status, or other life-style variables. In general, however, subjects were middle class, only three were married, and none reported occupational or living situations (such as night or swing shift work) which might be expected to cause aberrant hormone profiles. All reported regular menstrual cycles, with no significant difference in reported cycle length between groups (runners, 28.3 ± 0.6 days, versus controls, 28.3 ± 0.5 days). The two groups did not differ significantly in height (runners, 162.5 ± 2.2 cm, versus controls, 163.0 ± 1.3 cm) or age (runners, 30.7 ± 1.6 years versus controls, 29.3 ± 2.6 years). However, the runners were significantly heavier as a group (57.6 ± 2.6 kg versus controls, 50.7 ± 1.8 kg, $p < 0.01$, all levels of significance based on one-tailed t tests).

Each woman was provided with a set of polystyrene 12 × 75 collection tubes pretreated with sodium azide as a preservative. Samples of 2 to 5 ml of whole saliva were collected daily and stored at room temperature, the entire set being returned to the laboratory at the end of the month along with a record of menstrual dates and exercise. Samples were then frozen at −20° C until assayed. We have found a correlation of 0.89 ($p < 0.001$, $n = 20$) between progesterone values determined from matched frozen and unfrozen samples after 6 weeks. Subjects were requested to collect samples every day, but omissions occurred. For the 16 days preceding menstrual onset, the portion of the cycle analysed in this report, an average of 13.2 ± 0.7 sample days per cycle were collected from the runners

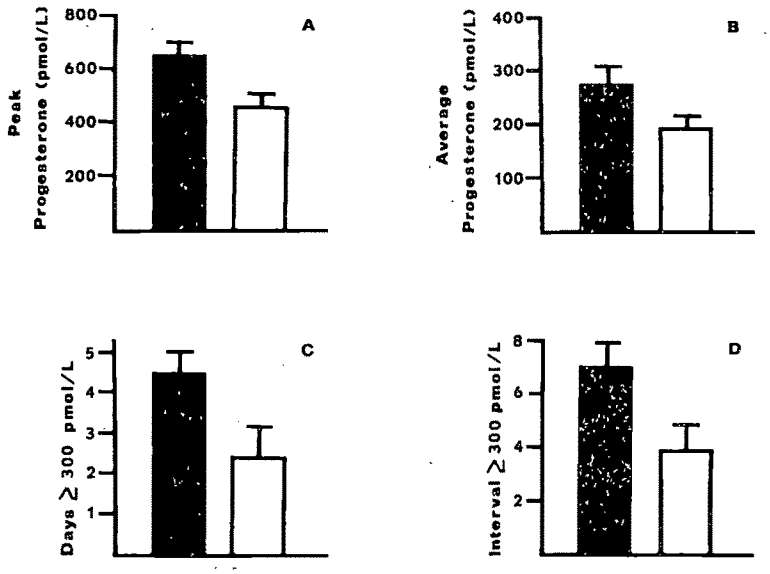


Fig. 2. *A*, Average peak luteal progesterone levels of the runners *(open bar)* and controls *(solid bar)*, $p < 0.01$. *B*, Average progesterone level over the 16 days before menstrual onset of runners and controls, $p < 0.05$. *C*, Average number of days with sample values ≥300 pmol/L among runners and controls, $p < 0.05$. *D*, Average interval between first and last observed value ≥300 pmol/L among runners and controls, $p < 0.05$. *Vertical lines* represent standard error. All p values based on one-tailed *t* tests.

and 12.5 ± 0.8 sample days per cycle from the controls ($p < 0.2$).

Samples were assayed for progesterone by radioimmunoassay, following a modified version of the method of Walker et al.[11] Assaying progesterone in saliva allows monitoring of ovarian function without undue disruption of a subject's normal routine. The high correlation between plasma-free and salivary progesterone levels has been well documented in a variety of experimental situations by many different investigators.[12] Because the relationship between plasma-free and salivary steroid levels is based on passive diffusion of free steroids across cell membranes,[13] there is no reason to expect that it would be affected by physiologic alterations in runners as a result of their exercise.

One milliliter of aqueous saliva was extracted twice in five volumes of diethyl ether with 1200 cpm of tritiated progesterone added as a tracer to quantify recovery, which averaged 80%. Extracted samples were dried under nitrogen and reconstituted in 1 ml of phosphate-gel buffer. Duplicate 400 μl aliquots were assayed in competition with 12,000 cpm of four-position tritium-labeled progesterone (New England Nuclear) for binding to a specific antibody, No. 337, generously supplied by Dr. Gordon D. Niswender. Reported cross-reactions of this antibody[14] indicated no need for chromatographic sample purification before assay. After 24-hour incubation at 4° C bound and free steroid were separated with dextran-coated charcoal, and radioactivity in the antibody-bound fraction was counted in a liquid scintillation counter. Two standard curves in triplicate were run with each assay. Results were quantified with a log-logit fit to the standard curve by programs supplied by Dr. David Rodbard. Assay sensitivity (smallest dose distinguishable from 0 with 95% confidence) was consistently <10 pg per assay tube, or approximately 50 pmol/L. Intra-assay and interassay variability were both below 13%.

Results

Fig. 1 illustrates the mean salivary progesterone levels, plus standard errors, determined for the runners and control subjects, aligned on the first day of menstruation. No difference in observed cycle lengths was found between the runners (27.5 ± 0.6 days) and the controls (27.0 ± 1.6 days). Nevertheless, the runners as a group displayed reduced levels of progesterone in the luteal phase of their cycles. Although the overall shape of the composite progesterone profile is strikingly similar in both groups, the runners have significantly lower peak progesterone levels (443 ± 58 pmol/L versus 655 ± 46 pmol/L, $p < 0.01$, Fig. 2, *A*), and lower average progesterone levels over the 16 days before menstrual onset (194 ± 24 pmol/L versus 287 ± 30 pmol/L, $p < 0.05$, Fig. 2, *B*). A sample value at least 2 SD above the follicular average of the controls, that is, >300 pmol/L, is operationally defined as indicating luteal activity. Such values are beyond the 95% confidence interval for follicular levels and thus represent statistically significant elevations of progesterone. Runners averaged fewer days of >300 pmol/L (2.5 ± 0.7 versus 4.5 ± 0.6 days, $p < 0.05$, Fig. 2, *C*) and a shorter

interval between the first and last days >300 pmol/L (4.0 ± 1.1 days versus 7.1 ± 1.0 days, $p < 0.05$, Fig. 2, *D*). Five of the 15 runners' cycles included no values >300 pmol/L and hence could not confidently be classified as ovulatory, whereas all the control cycles included values >300 pmol/L.

Although significant differences were observed in the progesterone levels attained by the two groups, no significant differences in the timing of the luteal phase were observed. The average day of peak progesterone secretion occurred 8.1 ± 0.7 days before menstrual onset in the runners versus 7.6 ± 0.5 days before menstrual onset in the controls. The first day of >300 pmol/L (for those cycles which attained this level) averaged 10.1 ± 0.7 days before menstrual onset for the runners versus 10.8 ± 0.8 days before for the controls, and the average interval between the first day >300 pmol/L and the peak day was 2.1 = 0.8 days for the runners and 3.2 ± 0.7 days for the controls.

It should be noted that our results do not address the physiologic mechanisms establishing the differences in observed salivary progesterone levels. Differences in salivary progesterone concentrations could result from differences in production rates, clearance rates, albumin binding, peripheral metabolism, or plasma volume.

Comment

Together with the results of previous studies, our results suggest that exercise may modulate female reproductive function in a continuous fashion, with mild exercise leading to mild impairment of function and more vigorous exercise leading to more extreme impairment. Low levels of exercise may first lead to a reduction in luteal progesterone levels, followed at increasing levels of activity by occasional and then frequent anovulatory cycles, shortened cycle length, oligomenorrhea, and finally amenorrhea. Only the more extreme end of this spectrum is visible based on menstrual characteristics, whereas the mild end requires regular monitoring of hormone profiles for detection.

Although clinical evidence has linked luteal phase defects with reduced fecundity,[15,16] it is not clear to what extent the level of progesterone suppression documented in this study may place implantation and early gestation in jeopardy. Nevertheless, the fact that such an effect can be observed at relatively low exercise levels, together with the fact that a growing number of women in their reproductive years regularly engage in recreational running and similar forms of aerobic exercise, renders the issue a matter of some public health concern.

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Are preoperative antibiotics helpful in abdominal hysterectomy?

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An open, randomized study to compare the effectiveness of triple-dose cefoxitin with the effectiveness of cephradine-tinidazole and with the result of nontreatment in patients undergoing abdominal hysterectomy was conducted from July 1983 to July 1984. One hundred ninety-nine patients were enrolled: 66 untreated control patients, 69 patients in the cephradine-tinidazole–treated group, and 64 patients in the cefoxitin-treated group. No significant differences were present in relation to age, pathologic condition, length of operation, blood loss, or length of hospital stay. That patients in both treatment groups had a lower incidence of postoperative febrile morbidity was not statistically significant. (AM J OBSTET GYNECOL 1986;154:1004-8.)

Key words: Preoperative antibiotics, hysterectomy

Hysterectomy is now one of the most frequently performed operations in Australia, and still 30% of women suffer morbidity resulting from operative site infection. This takes the form of abdominal wound infection, vaginal cuff infection, pelvic cellulitis, or pelvic abscess.

Various strategies, including systemic antibiotic prophylaxis,[1, 2] T-tube suction drainage of the retroperitoneal space,[3, 4] and topical irrigation of the surgical field with an aerosol antibiotic,[5] have been used effectively to reduce the incidence of pelvic cellulitis after vaginal hysterectomy. However, strategies to reduce morbidity after abdominal hysterectomy are not as well investigated or proved. In the Royal Hospital for Women, where approximately 75% of all hysterectomies are performed abdominally, the frequency of significant febrile morbidity is 32.7%, with 62% of all patients receiving antibiotics after hysterectomy. However, the effectiveness of antibiotic prophylaxis in reducing infection after abdominal hysterectomy is not well established.

Much of our knowledge of the cellular events leading to tissue infection is based on the thoughtful work of general surgeon John F. Burke,[6, 7] who concluded that there was a limited period when the operative site was receptive to the beneficial affects of antibiotic prophylaxis. He noted that the biochemical interaction between bacteria and host defenses began immediately when the organisms were inoculated, and to be effective in suppressing infection, antibiotics have to be given before the bacteria are introduced into the tissues. These original experiments have been verified by other studies.[8, 9]

Choice of antibiotic

Cefoxitin is a semisynthetic cephamycin antibiotic that is highly resistant to degradation by the β-lactamase enzymes produced by bacteria. It is active against all the clinically important gram-negative facultative bacteria except *Pseudomonas* and many *Enterobacter* species, as well as clinically important anaerobic organisms such as *Bacteroides fragilis*. It is inactive in vitro against most enterococci.

Cephradine is a semisynthetic cephalosporin antibiotic. Its range of activity is similar to that of cefoxitin except that cephradine has no activity against anaerobes, especially *B. fragilis*. This drug was chosen as the currently available intravenous cephalosporin in our hospital. No studies have been performed on cephradine specifically to determine whether the minimal inhibitory concentration is reached in pelvic wound fluid for expected contaminating flora. There is considerable variation within cephalosporins, and one cannot extrapolate from one to the other.[10]

Tinidazole is a nitroimidazole derivative that is claimed to be more bactericidal against *B. fragilis* than metronidazole[11] and to give higher and longer-lasting serum concentrations.[12, 13] In addition, 113 strains of anaerobic bacteria of the *Bacteroides, Peptococcus, Peptostreptococcus,* and *Clostridia* species have been shown to be sensitive to tinidazole.[14, 15]

Thus it seems that either cefoxitin or tinidazole-cephradine given preoperatively should cover the possible infective organisms and provide suitable prophylaxis against infection in pelvic tissues following abdominal hysterectomy.

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Table I. Preoperative factors with influence on incidence of infection

	Control	*Tinidazole*	*Cefoxitin and cephradine*
Average blood loss for all operations	434 ± 230	488 ± 260	443 ± 250
Average operating time for all operations (min ± SD)	76 ± 27	82 ± 27	82 ± 23

Table II. Pathologic conditions found

	Control		*Tinidazole and cephradine*		*Cefoxitin*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Fibroleiomyoma	33	50.0	29	42.0	29	45.00
Adenomyosis	18	27.0	11	15.9	13	20.00
Endometrial polyps	4	6.0	4	5.7	2	3.10
Endometrial hyperplasia	4	6.0	3	4.4	6	9.30
Endometriosis	7	10.6	4	5.7	2	3.10
Grade 3 cervical intraepithelial neoplasia	—	—	—	—	1	1.60
Adenocarcinoma of endometrium	4	6.0	2	3.3	4	6.75
Squamous carcinoma of cervix (invasive)*	1	1.5	10	16.0	3	4.60
Previous pelvic inflammatory disease	4	6.0	7	10.0	6	9.30
Other malignancies (ovary, fallopian tube, sarcoma)	1	1.5	2	3.3	4	6.25
No pathologic condition	9	13.6	6	8.6	5	7.80

*Excluded from further analysis.

Material and methods

An open, randomized study designed to compare the effectiveness of triple-dose cefoxitin with the effectiveness of cephradine-tinidazole and with the result of nontreatment in patients undergoing abdominal hysterectomy was conducted from July, 1983, to July, 1984, at the Royal Hospital for Women, Paddington, Australia. During this time, all patients undergoing hysterectomy were considered eligible for the protocol. Patients who had penicillin allergy, cephalosporin intolerance, or renal infection or who were currently taking antibiotics or requiring antibiotics for preexisting lesions (such as valvular heart disease) were automatically excluded from selection.

The patients were assigned to various treatment arms according to a predetermined randomized sequence. The attending physician was generally unaware of the medication given, and the assignment was performed by us. Treatment was organized as follows.

Those patients assigned to the cephradine-tinidazole group received oral tinidazole no greater than 12 hours before surgery (and generally on retiring): those on the morning list received the antibiotic on the evening before the operation, and those on the afternoon list received the antibiotic at 6 AM on the day of operation. One gram of cephradine was given intravenously as a bolus at induction of anesthesia. Those patients receiving preoperative cefoxitin received it as a 2 gm intravenous bolus injection at induction of anesthesia and at 6 and 12 hours postoperatively.

Hysterectomies were performed either by the senior medical officers at the hospital or by registrars in training supervised by a senior member of staff. Nearly all operations were performed with the patients under general anesthesia.

Table III. Procedures performed concurrently with total abdominal hysterectomy

	Control	*Tinidazole and cephradine*	*Cefoxitin*
Appendectomy	1	—	2
Retropubic urethropexy	2	—	1
Unilateral or bilateral adnexectomy	23	30	24

In the operating theaters, wound preparation of the patients was conducted according to the preference of the operating surgeon. It consisted of either povidone-iodine or 0.5% alcoholic chlorhexidine for skin preparation. All patients were catheterized before surgery. The duration of catheterization was not taken into account. The vulva and vagina were cleansed with 0.1% aqueous chlorhexidine. Alcoholic crystal violet was used to mark the vaginal vault.

Oral temperatures and other vital signs were taken every 4 hours postoperatively until the patient was discharged from the hospital.

Appropriate culture specimens were taken from the sites most likely to be infected as judged from the clinical evaluation. Specimens for other routine investigations, including vaginal swab and midstream urine or catheter specimen, were taken where indicated. Vag-

Table IV. Postoperative complications for all procedures

	Control		*Tinidazole and cephradine*		*Cefoxitin*	
	n	%	*n*	%	*n*	%
Total no. of patients	65		59		61	
Febrile morbidity	21	32.3	13	22	15	25
Patients with temperatures >38° C on more than two occasions	13	20	6	10	7	11.6
Proved urinary tract infection	5	7.6	—	—	1	1.6
Wound infection	3	4.5	—	—	—	—
Additional antibiotics	37	56.9	27	47	25	41.6
Hospitalization days	8.0 ± 1.5		7.9 ± 1.9		8.3 ± 1.8	

inal cuff specimens were not obtained unless there was discrete loculation of serosanguineous or purulent fluid at the vaginal apex.

Follow-up. Early follow up consisted of routine postoperative care and close attention to physical findings. The care was performed by the attending physician and his registrars (not by us). With any suspicion of infection, cultures of the involved sites were grown. Positive physical findings of signs of inflammation were noted on the hospital charts.

Late follow-up was done because some postoperative pelvic infections are insidious. A report was received from each surgeon after the patient had been examined at the postoperative visit. All infections not noted in the hospital should have become evident within this time. After this examination the study was terminated with respect to each patient.

The following criteria were used to define infection-related morbidity: (1) febrile morbidity, determined by two oral temperatures greater than 38° C taken at least 4 hours apart (excluding the day of surgery); (2) pelvic (vaginal cuff) cellulitis, determined by persistent temperature elevation in association with abdominopelvic pain, vaginal cuff tenderness, and localized pelvic peritoneal irritation; (3) pelvic (vaginal cuff) abscess, demonstrated by discrete loculation of purulent fluid at the apex of the vaginal vault and by drainage of infected material on probing of the suture line; (4) urinary tract infection, with the diagnosis based on clinical symptoms suggesting cystitis or pyelonephritis and confirmed by bacteriologic culture; (5) wound infection indicated by warmth, erythema, induration, pain and/or tenderness, as well as overt purulent drainage at the site of the surgical excision; and (6) the need for additional antibiotics.

Results

One hundred ninety-nine patients were enrolled in the study. Sixty-six of these patients were control subjects. Sixty-nine patients received tinidazole and cephradine, and 64 patients received cefoxitin. The average age of the control group was 43.2 years, that of patients who received tinidazole and cephradine was 44.1 years, and that of the cefoxitin group was 45.1 years. These differences are not statistically significant. The groups were not analyzed with respect to racial composition, gravidity, parity, or socioeconomic class. Eighty percent of patients in all groups were premenopausal. There is no significant difference between duration of operation and intraoperative blood loss (Table I).

Each group had comparable pathologic conditions (Table II).

Table III shows that the three groups had similar additional surgical procedures performed at the time of hysterectomy.

Table IV shows the postoperative complications experienced by the three groups.

Patients in both treatment groups had lower incidences of postoperative temperatures (as defined above), of proved urinary tract infection, and of wound infections (Table IV). The postoperative use of antibiotics was marginally reduced. The duration of hospitalization was not reduced. As shown by a χ^2 test, none of these differences are statistically significant. There were no patients in any group who had positive blood cultures, septic shock, or septic pelvic thrombophlebitis. There were only three patients—two control subjects and one treated patient—who required readmission for management of delayed sepsis. One control patient developed a wound abscess and a pelvic abscess and subsequently developed a pelvic hematoma. Another control patient developed a large, infected wound hematoma that required evacuation. One of the cefoxitin patients developed a wound sinus from which *Pseudomonas* was cultured. These three problems were late complications. These complications are not statistically significant. Several patients developed vault granulation tissue that required treatment, and another patient required resuturing of the vaginal vault. In this survey there is no statistical difference between patients treated preoperatively with tinidazole and cephradine and those treated preoperatively with triple-dose cefoxitin. In all, the incidence of significant infections or complications of 1.5% is exceedingly low.

Comment

There are at least 18 different reports on prophylactic antibodies in abdominal hysterectomy. Authors of twelve articles[4, 16-26] found that they were beneficial, and the authors of six articles[14, 27-31] did not.

In certain instances, antibiotics have been continued for 4 or 5 days following surgery. Such an extended use of antibiotics constitutes a therapeutic rather than a prophylactic regimen. In fact, the continuation of antibiotics up to 12 hours after operation, as performed in this trial, was requested by the suppliers of cefoxitin and is not consistent with Burke's original findings.[6, 7] Many investigations have failed to show that an extended course of antibiotics is significantly more effective than preoperative antibiotics. Moreover, an extended course of antibiotics does not render the operative site bacteria free.[24]

Another problem in comparing results is that there are different criteria for the definition of febrile morbidity, with cutoff temperatures of 37.8°, 38°, and 38.3° C being used in different series. Ledger and Kriewall[32] have proposed a "fever index" as an evaluation of these infections. This fever index is the number of febrile degree-hours above 99° F, excluding the day of surgery. This definition has not been used here: however, a crude estimation of the amount of fever has been performed: 32.3% of control subjects had two or more temperatures greater than 38° C, and 20% of patients had more than two temperatures greater than 38° C, with an average of 4.6 temperatures greater than 38° C. In the tinidazole-cephradine group, 22% met the criteria of standard febrile morbidity and 10% had an average of four temperatures at or greater than 38° C. The corresponding figures for cefoxitin are 25% and 11.6%, with an average of 3.7 temperatures. However, when the figures were analyzed with the χ^2 test, they were not statistically significant.

Febrile morbidity is not an accurate measure of treatment outcome because not all patients with febrile morbidity had overt clinical infections. However, since most practicing gynecologists within this study group use morbidity as an indication to prescribe antibiotics, the reduction of the temperature itself reduces the postoperative use of antibiotics; χ^2 analysis shows that this reduction in antibiotic use is not statistically significant. There was a great reluctance to perform a white blood cell count to determine whether significant infection was present. It seems apparent, from the rate of postoperative use of antibiotics, that a persistent low-grade temperature, irrespective of physical findings, is equated with operative site infection if urinary tract and wound infections have been excluded. Vaginal cultures were rarely grown. This attitude can be justified by the information acquired by Ohm and Galask.[27] Although this response is not intellectually satisfactory, practically it does not appear to be associated with any obvious harm to the patient.

In conclusion, on the basis of the present data and our literature review, the following conclusions appear to be justified:

1. Limited preoperative antibiotics appear to reduce the incidence of urinary tract infection after abdominal hysterectomy. The incidence (7%) is very low in the control group. Moreover, lower urinary tract infections rarely are a cause of serious morbidity in gynecologic patients, and the infections respond rapidly to oral antibiotics.

2. Prophylactic or preoperative antibiotics have not consistently reduced the incidence of pelvic cellulitis in other trials, nor do they appear to have markedly reduced the incidence in this survey.

3. Although there is a 10% reduction in the incidence of febrile morbidity and a 10% to 15% reduction in the use of postoperative antibiotics, these results are not statistically significant.

4. The incidence of major pelvic abscesses is so infrequent that a significant difference between control and drug regimens was not apparent.

Therefore, in patient populations such as ours, in which the incidence of significant postoperative febrile morbidity is low and serious sequelae of operative site infection are rare, the prevention of this possible outcome is not an obvious justification for the routine use of preoperative antibiotics.

The further rationale for use of preoperative antibiotics is the prevention of wound infection. However, the incidence of significant wound infection in our study group is so low that it does not comprise an adequate justification for the use of preoperative antibiotics.

Despite the apparent lack of established benefits of systemic antibiotic prophylaxis, however, antimicrobial agents should be given to a patient population with an inherently high risk of sepsis. This criterion does not appear to apply to our population, nor is it possible to identify a subset of our population (excluding patients with malignant disease) that may benefit from such prophylaxis. Indeed, it can be argued that antibiotic prophylaxis may, in fact, be harmful in that it may exert unfavorable selective pressure on the microbiologic flora and cause untoward side effects in selected individuals. Broad-spectrum antibiotics, such as cefoxitin, may best be reserved for treatment of acute infection.

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Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods

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Middle-aged women are becoming increasingly interested in aerobic exercise. For exercise to be meaningful, training needs to be performed at 70% to 85% of $\dot{V}O_{2max}$ for 20 minutes, three times weekly. The graded exercise test is the standard method used to determine maximum oxygen uptake, but this test is impractical for use in clinical practice. A cross-sectional study evaluated 163 women between 35 and 75 years of age by graded exercise test and compared the result with a matched group of 121 women tested by bicycle ergometer (predicted maximum oxygen uptake). Bicycle testing and the graded exercise test had a similar range of values; this was confirmed by 29 climacteric women performing both tests ($r = 0.789$). Menopausal status has no effect on cardiorespiratory fitness: the predicted maximum oxygen uptake of age-matched menstruating women was 27.4 ± 6.3 ml/kg/min and that of nonmenstruating women was 25.3 ± 4.2 ml/kg/min ($p > 0.05$). Bicycle ergometry can thus be used as a screen to determine the cardiorespiratory fitness status of climacteric women. (Am J Obstet Gynecol 1986;154:1009-13.)

Key words: Cardiorespiratory fitness, climacteric, maximal $\dot{V}O_2$, predicted $\dot{V}O_{2max}$, exercise prescription

It has become increasingly apparent that cardiorespiratory fitness may play an essential role in preventing cardiovascular disease and in controlling many of its risk factors. Today heart disease is the leading cause of death in the United States, affecting both men and women. In women it is most prevalent after the menopause; postmenopausal women have three times the cardiovascular disease incidence of premenopausal women.[2]

The current "fitness craze" has encouraged many people to begin programs of regular exercise, although these are primarily unsupervised and inconsistent in nature. The American College of Sports Medicine, in establishing guidelines[3] for exercise programs in adults, suggests that for individuals 35 years of age and older (and symptomatic individuals under 35) a medical evaluation be given before any major change in exercise habits. This evaluation should include a comprehensive medical history, including a personal and family history and current health habits such as smoking, medications, diet, etc. A 12-lead resting electrocardiogram and monitoring of blood pressure is also suggested, followed by an electrocardiogram-monitored graded exercise test. The graded exercise test can be used to measure the functional capacity of the patient and assess cardiorespiratory fitness and can serve as a basis for exercise prescription.

There are many protocols for graded exercise testing, all of which accomplish essentially the same thing, although some may be more suitable for a given population or a particular setting than others.[4] A graded exercise test is classified as either maximal or submaximal. A maximal test involves imposing progressively increasing workloads on the exercising subject until exhaustion or significant signs of intolerance to the activity are demonstrated. This type of test is most often used for direct measurement of maximum oxygen uptake ($\dot{V}O_{2max}$), the most accurate assessment of cardiorespiratory fitness.[5] This test, which requires the collection and analysis of expired air samples, is usually performed in a laboratory and is not well suited to use in everyday clinical practice. Submaximal testing, on the other hand, is more ideally suited to the practicing physician and his patients. A bicycle ergometer is commonly used; the patient rides against a progressively increasing workload until a given heart rate is attained, from which the workload is calculated. The point at which this criterion is met can be used to estimate $\dot{V}O_{2max}$.[6]

Several studies have shown that predicted oxygen uptake testing correlates well with observed maximal testing when corrected for age,[7] but none of these stud-

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Table I. Description of subjects

Age group	*Measured (group 1)*			*Estimated (group 2)*		
	Height (cm)	*Weight (kg)*	*Age (yr)*	*Height (cm)*	*Weight (kg)*	*Age (yr)*
35-44 yr		n = 13			n = 30	
Mean	60.3	162.3	39.3	61.9	164.7	40.5
SD	9.9	6.9	3.9	8.6	6.3	4.9
45-54 yr		n = 60			n = 47	
Mean	61.0	161.8	50.8	64.2	164.6	49.8
SD	7.2	6.3	2.5	8.6	6.3	4.9
56-65 yr		n = 70			n = 24	
Mean	61.2	161.0	58.7	59.1	160.5	59.3
SD	7.4	5.6	3.1	8.8	5.5	2.8
66-75 yr		n = 20			n = 10	
Mean	59.1	158.2	69.4	63.7	160.0	68.7
SD	7.6	5.5	3.1	6.4	5.6	2.3

Table II. Mean estimated oxygen uptake values and hormonal status (±SD) of menstruating and nonmenstruating women in subgroup 46 to 55 years

Parameter	*Menstruating (n = 28)*	*Nonmenstruating (n = 30)*
Estimated $\dot{V}O_{2max}$ (ml/kg/min)	27.4 ± 6.3	26.3 ± 4.7
Luteinizing hormone (mIU/ml)	23.5 ± 3.6	62.8 ± 3.5
Follicle-stimulating hormone (mIU/ml)	12.6 ± 2.6	55.7 ± 3.5
Estrone (pg/ml)	107.5 ± 11.5	62.9 ± 3.9
Estradiol (pg/ml)	146.2 ± 18.7	19.5 ± 3.5

ies have examined climacteric women. This investigation was concerned with three main issues: (1) We compared the range of values derived from women aged 35 to 75 performing maximal $\dot{V}O_2$ testing with values in a similar group who were evaluated for $\dot{V}O_{2max}$ by means of a submaximal bicycle test. (2) We compared maximal and submaximal values by having the same subject perform both tests. (3) Since the onset of the menopause has been linked with an abrupt change in the cardiorespiratory fitness of women,[8] a subgroup of women was studied to see whether the menopause per se had any effect on cardiorespiratory function.

Methodology

All of the subjects were evaluated at the Center for Climacteric Studies Wellness Laboratory after informed consent had been obtained and a full physical examination performed. The subjects reported to the laboratory between 7:00 AM and 9:00 AM after a 12-hour fast and having had approximately 8 hours of sleep the previous night. The subjects were instructed to abstain from nicotine, caffeine, and alcohol and were asked not to exercise on the day preceding the test.

One hundred sixty-three healthy sedentary women (group 1) between the ages of 35 and 75 years were accepted as subjects for maximal testing after being screened for cardiovascular normalcy by a 12-lead electrocardiogram stress test and physical examination. The $\dot{V}O_{2max}$ was elicited by a modified Balke and Ware[9] treadmill protocol. The speed of the treadmill remained constant at 3.0 mph (versus 3.3 mph) throughout the test, while the elevation increased 1% each subsequent minute until either the subject declared fatigue or $\dot{V}O_2$ values failed to increase with increasing workload. Each subject's $\dot{V}O_{2max}$ was directly assessed by means of a Beckman Metabolic Measurement Cart. An electrocardiograph was used to monitor lead V_5 during the test; heart rates were calculated from the electrocardiogram printout obtained every minute.

One hundred twenty-one women (group 2) who met the same criteria were selected for participation in a study with the use of submaximal testing to predict $\dot{V}O_{2max}$. Each subject also underwent a complete physical examination, and a resting electrocardiogram was obtained. A submaximal bicycle ergometer test, as described by Åstrand,[6] was used to estimate $\dot{V}O_{2max}$.

The physical characteristics of the subjects who participated are described in Table I. They were closely matched as regards their mean age height, and weight ($p > 0.05$).

To examine the reproducibility of the tests in women evaluated by both the treadmill and ergometer techniques and to differentiate between the effects of menopause and aging, two further subgroups were chosen. Twenty-nine women participating in an ongoing exercise program were selected to have both the treadmill and ergometer tests (group 3). Their mean age was 55.6 ± 9.1 years. All were postmenopausal and had been trained for a minimum of 3 months. Ten were treadmill walkers, nine stationary bicyclers, and 10 exercised on Nautilus (muscle-strengthening) equipment. They were evaluated as indicated above before entry into the study and had a treadmill and ergometer test on two separate occasions. The interval between testing was <1 month. The order of testing was randomly selected.

To test for the effect of the menopause on cardiorespiratory fitness, women aged 45 to 55 from the ergometer-tested group were divided according to whether or not they were still menstruating. Premenopausal or postmenopausal status was confirmed by hormonal analysis and by menstrual pattern; postmenopausal women were required to have been amenorrheic for at least 1 year. The hormonal analysis was per-

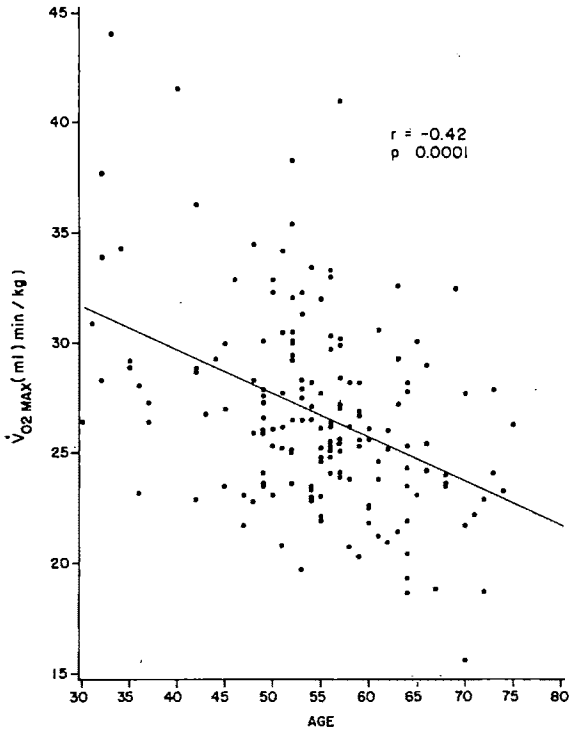


Fig. 1. Maximal oxygen uptake measured by a modified Balke and Ware treadmill procedure in 163 healthy climacteric women.

formed from blood samples obtained between 8:00 AM and 9:00 AM, according to methods described in detail elsewhere.[10] As reflected in Table II, serum luteinizing hormone and follicle-stimulating hormone levels were significantly higher in the nonmenstruating women ($p < 0.0001$), and the respective estradiol and estrone levels were significantly lower ($p < 0.0001$). The menstruating women were slightly younger (48.7 ± 0.4) than their amenorrheic peers (52.2 ± 0.4), but the difference was not statistically significant.

The data obtained were expressed as means and analyzed by an independent *t* test to determine whether significant differences existed between the groups.

Results

Table III describes the observed (measured) and the estimated $\dot{V}O_{2max}$ data of the two groups. Subjects were grouped by age and manner of assessment. There were no significant differences in mean $\dot{V}O_{2max}$ ($p > 0.05$) in any age group between those subjects who underwent maximal testing (group 1) and those who participated in submaximal testing (group 2). Similarly, the range of values for subjects in both groups showed no significant differences. The only apparent difference between these two groups was seen in the rate of change in $\dot{V}O_{2max}$ in successive decades. The measured $\dot{V}O_{2max}$

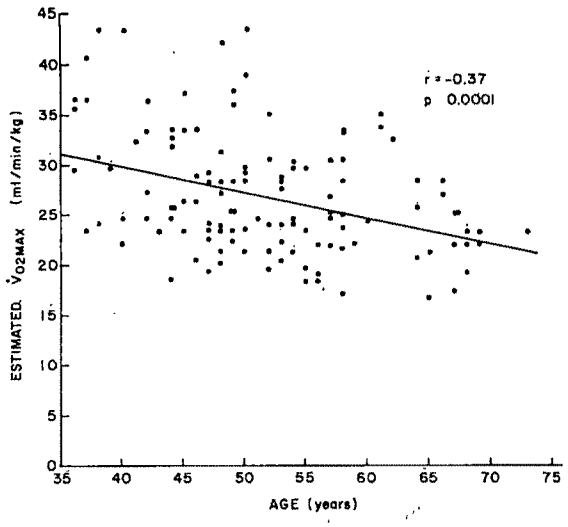


Fig. 2. Predicted maximal oxygen uptake determined by the Åstrand ergometer method in 121 healthy climacteric women.

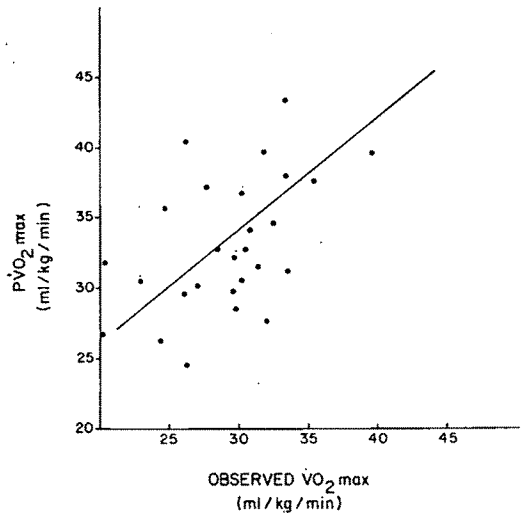


Fig. 3. Correlation between predicted and observed $\dot{V}O_{2max}$ derived by 29 women exercising on an ergometer ($P\dot{V}O_{2max}$) and treadmill testing (*observed* $\dot{V}O_{2max}$) ($r = 0.789$).

group showed an average decrement of 5.5% of $\dot{V}O_{2max}$ with each succeeding decade between ages 35 and 75 (Fig. 1), while the estimated $\dot{V}O_{2max}$ group lost an average of 7.4% of aerobic capacity each decade (Fig. 2).

The measured $\dot{V}O_{2max}$ of the 29 exercising women (group 3) participating in both test modalities was 28.6 ± 4.9 ml/kg/min, and the predicted $\dot{V}O_{2max}$ (determined with the ergometer) 32.5 ± 5.3 ml/kg/min. When the latter result was calculated with the recommended Åstrand age-corrected factor,[7] the mean predicted $\dot{V}O_{2max}$ was 23.4 ± 4.9 ml/kg/min. This result correlated closely with the direct measured result (r value = 0.789) (Fig. 3).

Table III. Maximal oxygen uptake value by age group for measured and estimated testing

Age group	$\dot{V}O_{2max}$ (ml/kg/min)	
	Measured (group 1)	Estimated (group 2)
35-44 yr	n = 13	n = 30
Mean	29.0	29.9
SD	4.9	6.5
45-54 yr	n = 60	n = 47
Mean	27.4	27.7
SD	3.9	8.0
55-64 yr	n = 70	n = 24
Mean	25.9	25.3
SD	3.8	5.3
65-75 yr	n = 20	n = 10
Mean	24.2	23.3
SD	4.0	3.1

No significant difference was found in the mean estimated $\dot{V}O_{2max}$ of premenopausal and postmenopausal women of similar age (Table II). Those women still menstruating had a slightly higher mean $\dot{V}O_{2max}$ (27.4 ± 6.3 ml/kg/min) than their nonmenstruating counterparts (26.3 ± 4.7 ml/kg/min), but this difference was not significant ($p > 0.05$).

Comment

Physical exercise performed at activity levels <50% of maximum heart rate can be maintained for several hours; to achieve physical fitness and its associated benefits, training needs to be performed at 70% to 85% of maximum heart rate for at least 20 to 40 minutes three times per week. Training levels sustained in excess of this range could be harmful to individuals with asymptomatic cardiovascular disease. For sedentary individuals who wish to participate in meaningful exercise, the graded exercise test is as integral a part of screening a patient as is the medical history. The graded exercise test is used to determine patient response (heart rate, blood pressure, electrocardiogram) to increasing intensity of exercise and can be used to determine $\dot{V}O_{2max}$, a measure of cardiorespiratory fitness that is representative of the efficiency of the oxygen transport system.[5] The $\dot{V}O_{2max}$ (or an estimate of such a value) is thus an important key to determining a proper exercise program. Individualized prescriptive exercise programs can be designed by use of a percentage of this value for the intensity level at which the exercise program should be performed. It can also serve as a measure of the conditioning effect of training by the incorporation of periodic $\dot{V}O_{2max}$ retesting into the program.

There is a gradual loss of $\dot{V}O_{2max}$ with age of slightly less than 1% per year in sedentary individuals, which becomes more apparent after about age 50. The rate of decline in $\dot{V}O_{2max}$ is usually, as in this study, determined by cross-sectional sampling of healthy individuals of different ages.

Table IV. Guidelines for fitness assessment by maximal oxygen uptake of healthy women aged 30 to 70 years

Age group	$\dot{V}O_{2max}$ (ml/kg/min)				
	Poor	Fair	Average	Good	Excellent
30-39 yr	<20	20-27	28-33	33-44	45+
40-49 yr	<17	17-23	24-30	31-41	42+
50-59 yr	<15	15-20	21-27	28-37	38+
60-69 yr	<13	13-17	18-23	24-34	35+

Adapted from Exercise Testing and Training of Apparently Healthy Individuals: A Handbook for Physicians. ●●●, American Heart Association, 1972:15

Dehn and Bruce[11] in a longitudinal study of healthy men determined that the coefficient for the rate of change was twice that determined by cross-sectional data. The decline in $\dot{V}O_{2max}$ reflects a loss of functional capacity, which may be attributed in part to the natural deterioration of the aging process and partly to age-related decrease in activity. The decline in maximal oxygen uptake with age is less pronounced in men who are habitually physically active, as compared with inactive individuals.[11] However, a study of physically active Swedish women,[12] who were assessed 20 years after the initial test, noted a 19% reduction in the subjects' maximal oxygen uptake values: 47.6 ± 0.7 versus 38.4 ± 0.7 ml/kg/min. Given the cross-sectional nature of our study and the sedentary female population studied, the directly measured $\dot{V}O_{2max}$ loss of 5.5% per decade (group 1) is less than that expected. However, the result is consistent with the predicted values obtained in the matched group (group 2) evaluated by ergometry—7.4% per decade. The values obtained in our women who were directly measured are compatible with those obtained by others for sedentary middle-aged women.[13]

By notation of the plateau of oxygen uptake with intense and prolonged exercise on the graded exercise test, it is possible to accurately determine the "true" point of maximal oxygen uptake. Higher values for $\dot{V}O_{2max}$ are thus usually obtained with the treadmill test. Walking, as in the graded exercise test, is more compatible with everyday activities than cycling, which is a "foreign" activity to middle-aged women in the United States. The greater observed $\dot{V}O_{2max}$ in our trained subjects (28.58 ± 4.86 ml/kg/min) could be explained by these factors and was therefore an expected result. The graded exercise test correlated well ($r = 0.789$) with the ergometer-devised test, thus confirming the experience of others involving male subjects.[7]

Although a reduction in $\dot{V}O_{2max}$ occurs in both men and women, it has been suggested that the onset of menopause may contribute to the intensified decline in aerobic capacity that appears near age 50.[14] When 45- to 55-year-old women were divided into menstruating and nonmenstruating groups, no significant difference was seen in mean estimated $\dot{V}O_{2max}$ of these women, supporting recent research showing that the menopause per se does not play a significant role in the decline of $\dot{V}O_{2max}$ with age.[15] The functional loss in the perimenopause may be curtailed by a program of regular physical activity. Cowan and Gregory[15] reported that premenopausal women (mean age 41.3 ± 4.4 years) who trained for 9 weeks had a 12.1% improvement in $\dot{V}O_{2max}$, and postmenopausal women (mean age 56.7 ± 5.2 years) who trained for a similar period had a 19% improvement.

On the basis of the documented decline in both maximal and submaximal testing in our laboratory (Figs. 1 and 2), the guidelines for evaluating the fitness status of climacteric women, with the use of either method, are compatible with those published previously (Table IV). Guidelines such as these are useful when one is evaluating a patient before prescribing exercise. It can serve as a reference point for the physician and his patient to determine her fitness status relative to that of other women in her age group and to provide a baseline value from which to start a program and measure improvement. Bruce et al.[16] recently reported that 63% of their patients attributed a change in one or more risk factors and health habits to a graded exercise test; persons with an abnormal result were motivated the most. Exercise testing may thus play an important part in stimulating women to participate in and continue with meaningful exercise.

In conclusion, a thorough medical examination, which includes testing of cardiorespiratory function, is advisable for patients adding regular exercise to their daily routine. Such an evaluation will ensure safer participation and will add ease and consistency to the monitoring of their progress. In addition, periodic examinations that show progress may help to establish and maintain greater patient rapport and encourage the compliance needed to achieve physical fitness.

The data from this comparative study indicate that bicycle testing for the estimation of $\dot{V}O_{2max}$ will elicit a range of values similar to those derived from maximal testing with a treadmill and the direct measurement of expired gases. Submaximal testing can thus be used as a screen to determine the cardiorespiratory fitness status of climacteric women. It is the method best suited for use in clinical practice because of its ease of administration. This is true irrespective of the menopausal status or fitness of the patients concerned. Patients at high risk for cardiovascular disease and those classified as having fair-poor fitness (as measured by ergometry) require more detailed evaluation before they embark on a prescribed exercise program.

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Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant

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Vitamin E and β-carotene are two important natural antioxidants. However, the mean (± SD) serum concentrations of β-carotene in the cord blood of term (17.9 ± 4.4 μg/dl) and preterm (14.04 ± 4.7 μg/dl) infants are one eighth the concentration in the maternal serum (131 ± 43 μg/dl). Likewise the serum concentrations of vitamin E in the term (0.31 ± 0.09 mg/dl) and preterm (0.29 ± 0.08 mg/dl) infants are one-third the concentration in the maternal serum (0.97 ± 0.16 mg/dl). Human breast milk, particularly colostrum, contains very high concentrations of both vitamin E (3.28 ± 2.93 mg/dl) and β-carotene (213 ± 166 μg/dl). Thus the breast-fed, term infant attains serum levels of both vitamin E and β-carotene comparable to those in the adult within 4 to 6 days of breast-feeding. This study shows that the seeming barrier in the fetus to access to the antioxidants vitamin E and β-carotene, in utero, is rapidly corrected and the substances are replenished postnatally through breast-feeding. This study therefore alludes to the possible role of breast-feeding in providing for the infant's defense against oxygen toxicity. (Am J Obstet Gynecol 1986;154:1014-7.)

Key words: Antioxidant, vitamin E, β-carotene, breast-feeding

Vitamin E and β-carotene are two important natural antioxidants. However, infants are born with significantly lower serum levels of vitamin E and β-carotene than the mother,[1-4] and the reason still eludes us. It has been postulated that the susceptibility of the premature infant to oxygen injury may be one reason for this deficiency.[5] As far as we know, no information is available on the natural restoration of the plasma levels of vitamin E and β-carotene in the infant postnatally. The purpose of this study was to determine, in a prospective design, the impact of breast-feeding on the restoration of the serum levels of vitamin E and β-carotene in a group of term infants.

Material and methods

A profile of the cord serum concentrations of β-carotene and vitamin E in paired maternal venous blood and cord blood was first obtained in 28 premature and 31 term infants. The gestational age of the infants was assessed by physical examination according to the method of Ballard et al.[6] Likewise, a profile of β-carotene and vitamin E concentrations in breast milk was determined. Breast milk was serially obtained from the first to the fifth day from 19 lactating mothers, five of whom had premature infants and 14, term infants. The mothers were taking regularly prescribed vitamin supplements for pregnancy. One mother further supplemented her daily diet with carrot juice.

The effect of feeding on the serum levels of vitamin E and β-carotene was studied in two groups of cesarean section–delivered term infants,* either breast-fed (N = 16) or formula fed (N = 8) with Similac 20 or Enfamil 20. Serum samples were obtained from cord blood and every other day from each group of infants from the second day to the day of discharge (days 4 to 6). Informed consent was obtained. The study protocol was approved by the Wayne State University Human Investigations Committee.

β-Carotene and total vitamin E in the serum (or milk) was determined by the spectrophotometric method of Hashim and Struttringger,[7] modified as follows: Into a small centrifuge tube, add the following by Lang Levy pipette: 200 μl of serum (or milk), 200 μl of absolute ethanol, and 400 μl of heptane. Whirl into a vortex for 10 minutes. Centrifuge at 3000 rpm for 10 minutes. With a Lang Levy pipette, transfer 300 μl of the upper heptane layer to a microcuvette (International Crystal

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*This study was confined to term infants because an adequate oral intake of milk was essential to the study. The infants delivered by cesarean section were chosen because of their longer stay in the nursery.

Table I. Levels of β-carotene and vitamin E in maternal serum and cord blood of premature and term infants (mean ± SD)

	Term (N = 31)	*Premature (N = 28)*
Infant		
Gestational age (wk)	39.7 ± 0.6	33.9 ± 3.0
β-Carotene (μg/dl)	17.9 ± 4.42	14.04 ± 4.74*
Vitamin E (mg/dl)	0.31 ± 0.09	0.29 ± 0.08
Mother		
β-Carotene (μg/dl)	131.0 ± 43	120.6 ± 49.9
Vitamin E (mg/dl)	0.97 ± 0.16	0.91 ± 0.17

*$p < 0.01$ by Student's *t* test.

Laboratory, 1 cm light path and 0.2 cm width). In a Zeiss PMII spectrophotometer, read absorbance of β-carotene at 452 nm, using heptane as a blank. Add 40 μl of bathophenanthroline reagent (Sigma), 0.147% in absolute ethanol. Add 20 μl of $FeCl_3 \cdot 6\ H_2O$ reagent (Sigma), 0.040% in absolute ethanol. Cover and shake. Read absorbance at 532 nm for vitamin E as soon as the blank stabilizes. Calculate the concentration of β-carotene and vitamin E from standard curves constructed from pure β-carotene and vitamin E solutions (Eastman Chemical Co.). A correction for the absorbance of β-carotene at 532 nm is necessary for the calculation of the vitamin E concentration. In this spectrophotometric assay of β-carotene and vitamin E, no interference was noted from bilirubin or unsaturated free fatty acids.

Statistical analyses were done by paired or unpaired Student's *t* test and by regression analysis.

Results

Higher levels of β-carotene ($p < 0.01$), but not vitamin E, were found in the cord blood of term versus premature infants (Table I). No significant difference was noted in the maternal plasma levels of β-carotene and vitamin E of either the premature or the term infants. A large gradient in β-carotene and vitamin E concentrations was noted between maternal plasma and cord blood levels—a ratio of 8 : 1 and 3 : 1 for β-carotene and vitamin E, respectively.

Significant correlations ($r = 0.35$; $p < 0.01$) were found between the cord serum level of β-carotene and the infant's gestational age (Fig. 1) and between the β-carotene level ($r = 0.44$; $p < 0.01$) in the cord blood and that in the maternal blood (Fig. 2). No significant correlation was seen between the cord vitamin E level and either the infant's gestational age or the maternal vitamin E level, nor was there a correlation between levels of maternal vitamin E and β-carotene and the infant's gestational age.

Human milk, particularly colostrum, contained very high levels of vitamin E and β-carotene (Table II). Mean concentrations of these substances were highest in breast milk of 1 to 3 days, after which their concentrations rapidly diminished. In comparison, the two formulas analyzed (Enfamil 20 and Similac 20) had negligible concentrations of β-carotene and had levels of vitamin E similar to those found in human milk at 4 to 5 days of age. Human milk obtained from mothers who had term or preterm infants showed no significant difference ($p > 0.10$) in levels of β-carotene or vitamin E.

The effect of feeding on the serum level of β-carotene and vitamin E in the infants is shown in Fig. 3. A steady and significant ($p < 0.01$) increase in the serum concentration of both β-carotene and vitamin E occurred in breast-fed infants from day 2 onward. The formula-fed infants experienced a significant rise only in vitamin E levels and not in β-carotene levels, essentially as a result of the negligible content of β-carotene in the formula (Table II). Within the limited period of the study, 7 of the 15 breast-fed infants attained comparable adult serum levels (≥40 μg/dl) of β-carotene within 4 to 6 days of breast-feeding. Likewise, 12 of the 16 breast-fed infants had comparable adult levels (≥0.60 mg/dl) of vitamin E at days 4 to 6 of breast-feeding.

Comment

The data from this study confirm previously reported observations that the fetus has markedly lower serum levels of β-carotene and vitamin E in comparison with the mother.[1-4] The reason for this discrepancy has so far been unclear. Vitamins are transported across the placenta through the mechanism of a simple or facilitated diffusion, active transport, or pinocytosis.[8] Many factors, such as lipid solubility of the substrate and placental age, thickness, and surface area, may further modify the rate of transfer of these substances.[9] Still other factors may influence the fetal serum concentration, such as levels of carrier protein in the fetus, occurrence of reverse transport, placental and fetal metabolism of vitamins, and tissue storage in the fetus.[8] The majority of the water-soluble vitamins (vitamin B groups and vitamin C) are transported by active processes resulting in fetal concentrations of these vitamins two to five times higher than those on the maternal side.[10] In contrast, the serum concentrations of lipid-

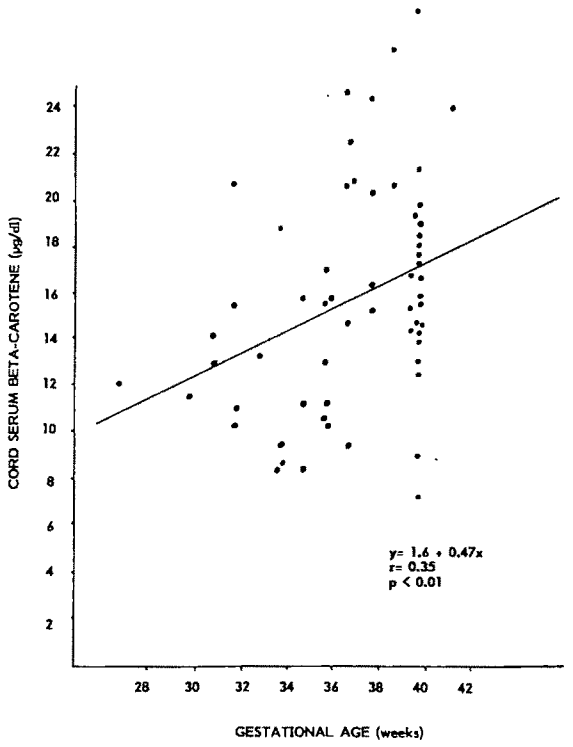


Fig. 1. Correlation between cord blood β-carotene level and gestational age.

soluble vitamins, vitamin E and β-carotene, in the fetus are generally less than the maternal levels.[1-4] Although this discrepancy may suggest such mechanisms as passive or facilitated diffusion, neither the actual mechanism of transport nor the factors affecting it are clear. For instance, megadose administration of vitamin E or β-carotene to mothers does not cause a commensurate rise in fetal serum levels,[2, 11, 12] as might be expected from a simple diffusion phenomenon. Whatever the reason, low serum levels of vitamin E or β-carotene in the fetus exist even among normovitaminemic mothers.[10-12] Thus it is reasonable to assume that the low serum levels of vitamin E and β-carotene in the fetus reflect a physiologic rather than a deficiency state.

Soon after birth, the situation changes. The breast-fed infants receive a high dose of both vitamin E and β-carotene in the breast milk, particularly in the colostrum. Consequently, as shown by this study, their serum β-carotene and vitamin E concentrations progressively increase, and within 4 to 6 days of breast-feeding, restoration to serum levels comparable to those in adults occurs in a significant number of the infants. Thus the seeming barrier to access to vitamin E and β-carotene in utero is rapidly corrected, and the two antioxidants are replenished postnatally through the breast milk. In contrast, the formula-fed infants do not show any increase in their serum β-carotene level. The reason is

Table II. Concentrations (mean ± SD) of β-carotene and vitamin E in human milk and in formula

	β-Carotene (µg/dl)	*Vitamin E (mg/dl)*
Human milk		
Day 1 (N = 14)	213.0 ± 166.6	3.28 ± 2.93
Day 2 (N = 20)	117.0 ± 112.0	2.74 ± 2.30
Day 3 (N = 17)	120.0 ± 63.4	2.09 ± 1.44
Day 4 (N = 10)	50.4 ± 19.9	1.03 ± 0.50
Day 5 (N = 4)	39.5 ± 34.7	0.45 ± 0.33
Formula		
Similac 20 (N = 3)	Negligible*	0.91 ± 0.03
Enfamil 20 (N = 3)	Negligible	1.16 ± 0.04

*Absorbance within matching error of cuvettes.

that currently available formulas, unlike colostrum, do not contain any measurable amount of β-carotene (Table II). On the other hand, since formulas are fortified with vitamin E, formula-fed infants show a progressive increase in their serum vitamin E levels similar to those achieved among breast-fed infants. It is of interest to note that although breast milk contains a higher concentration of vitamin E than currently available formulas (Table II), the serum concentration of vitamin E attained in the breast-fed infant is not significantly higher than that seen in the bottle-fed infant (Fig. 3). The reason may relate to the bioavailability, absorption, and volume of distribution of vitamin E in the infant. For instance, it has been shown that to achieve and maintain a serum vitamin E concentration in the infant of 3 to 4 mg/dl, inordinately high doses of vitamin E (25 to 100 mg) have to be orally administered daily.[13]

Vitamin E and β-carotene are two important naturally occurring antioxidants in biologic systems: β-carotene functions as an avid singlet oxygen quencher,[14] and vitamin E protects principally against the peroxidation of lipids in cell membranes.[15] The antioxidant characteristics of these two substances have been involved or tested in various biologic and clinical studies.[13, 16] Therefore the access of the newborn infant to high concentrations of both antioxidants, vitamin E and β-carotene, in the breast milk alludes to an important role of breast milk—that of providing for the infant's defense against oxygen toxicity. This study, by design, was confined to a population of term infants. Although its results support the concept that breast-feeding provides for a replenishment of the antioxidant state in the newborn infant, it will be important to determine whether the same bioavailability of vitamin E and β-carotene occurs in the premature infant who is breast fed or otherwise given human milk, since the risk of the toxic effects of oxygen is particularly high in this group of infants.

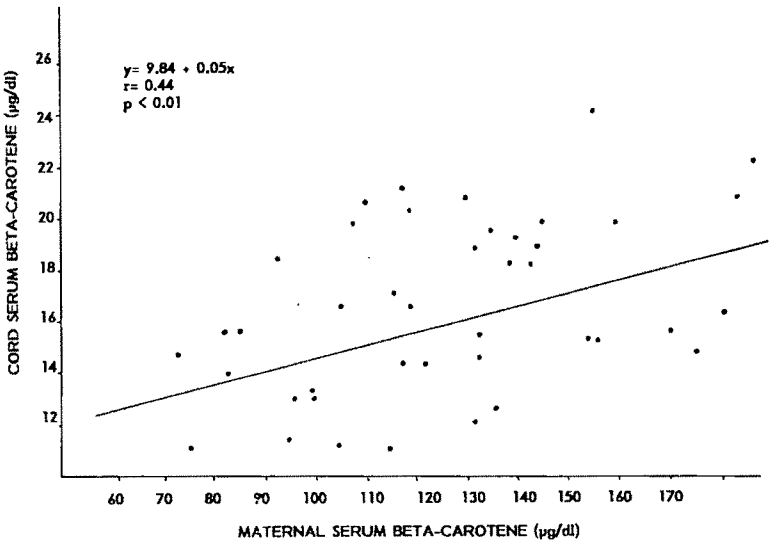


Fig. 2. Correlation between cord and maternal serum β-carotene levels.

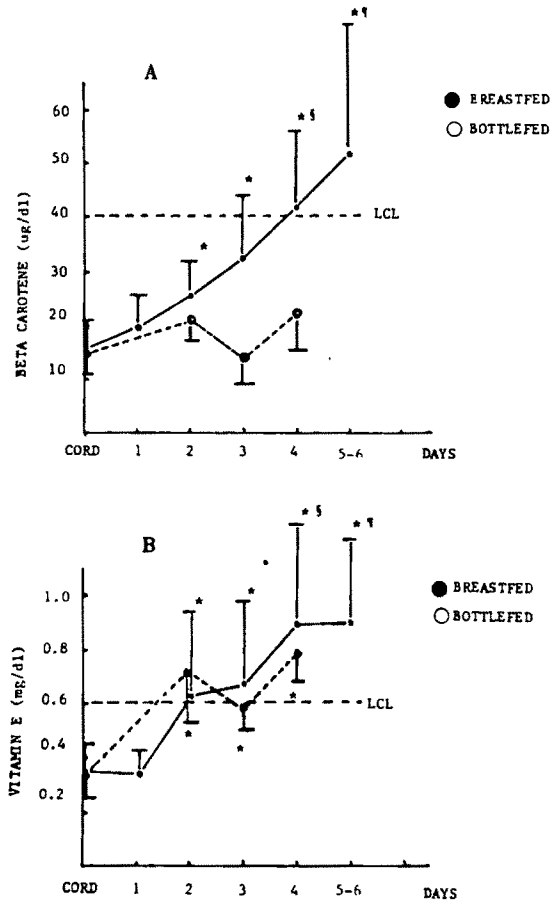


Fig. 3. Serum β-carotene *(A)* and vitamin E *(B)* levels from days 1 to 6 in breast-fed and bottle-fed infants. The lower 95% confidence limit *(LCL)* of the normal adult serum concentrations of vitamin E and β-carotene is indicated by the *dashed line* (reference 19). *$p < 0.02$ when compared with cord; §$p < 0.01$ when compared with day 2; ¶$p < 0.01$ when compared with day 3.

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Postpartum moods in men and women

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Twenty-one married couples, recruited from childbirth classes (mean age 29.6 years), were administered questionnaires measuring 20 different moods during the third trimester of pregnancy (prepartum period), during the postpartum period, and at 6 months after birth (follow-up period). In each questionnaire period individual questionnaires were filled out daily by both the mother and father for 10 consecutive days. The results indicated that the postpartum period, compared with the prepartum and follow-up periods, is an emotionally unique time but not a period marked by depression. The moods that were rated as being experienced more strongly by men and women during the postpartum period were associated with anxiety and concern for one's ability to cope such as "nervousness," "worried," "helpless," and "anxious" or positive emotions such as "enthusiastic" and "happy." It is concluded that men and women in this sample tend to experience the postpartum period in an emotionally similar way. (AM J OBSTET GYNECOL 1986;154:1018-23.)

Key words: Postpartum period, moods, women, men

The postpartum or puerperal period begins after the delivery of the child and ends approximately 6 weeks later when the reproductive tract returns to a normal nonpregnant condition. There is a wealth of literature concerning feelings of depression during the postpartum period. These feelings range from very mild "maternity blues" and "postpartum depression," which is characterized by a transitory state of tearfulness, depression, and anxiety, to rare cases of postpartum psychosis, which is a serious mental illness associated with the childbirth process and postpartum period.[1-5]

"Maternity blues" or "postpartum depression" has been reported for 20% to 89% of mothers, depending on the study.[4-7] Postpartum psychosis is a rarer phenomenon and has been reported as occurring in 3.0 to 3.6 per 1000 women.[8]

A variety of variables have been associated with postpartum depression, including age, a history of premenstrual tension, concern about the feeding habits of the baby, high neonatal risk, number of previous children, whether the woman is employed outside the home, and social, cultural, and personal expectations.[4, 5, 9, 10] The exact cause of postpartum depression is unknown, but several studies suggest it may have a hormonal basis associated with the dramatic changes in estrogen and progesterone levels during pregnancy and at the time of birth.[2, 4, 11, 12]

In spite of the fact that postpartum depression has traditionally been associated with the biologic mother, some studies have reported incidences of postpartum depression in other family members.[4, 7, 13-15] Atkinson and Rickel[15] found that 13% of their sample of middle-class men reported having felt depressed at some point in time during the 8 weeks after the birth of their first baby, and this was the same as the prepartum percentage. The men's depression was due, in part, to the fact that they viewed the baby in a less positive way than they anticipated they would before the birth of the baby. Zaslow et al.,[7] studying a sample of 57 middle-class families after the birth of their first child, found that 62% of the fathers and 89% of the mothers reported that they had experienced postpartum depression at some point in time in the 4 months since the birth of their babies. No data were provided for levels of depression for men and women in the period before the birth of the child.

The purpose of the present study was to further investigate mood changes in both men and women during the postpartum period. A group of expectant parents was studied at three points in time: prepartum period, postpartum period, and 6 months after the birth of the baby. During these three time periods both parents were asked to fill out questionnaires regarding 20 different moods to ascertain if the postpartum period produced markedly more negative moods than the prepartum and 6-month follow-up periods.

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Table I. Means, SDs, F values, and p values for time period effects

Variable	*Prepartum*	*Postpartum*	*Follow-up*	*F*	*p*
Nervous					
Mean	0.50	0.66	0.41	9.92	<0.005
SD	0.35	0.45	0.43		
Self-confident					
Mean	2.34	2.30	2.51	5.37	<0.009
SD	0.69	0.73	0.67		
Worried					
Mean	0.60	0.79	0.50	7.45	<0.002
SD	0.39	0.55	0.52		
Angry					
Mean	0.35	0.21	0.34	5.46	<0.008
SD	0.30	0.25	0.47		
Energetic					
Mean	1.92	1.88	2.14	5.34	<0.009
SD	0.71	0.74	0.68		
Enthusiastic					
Mean	1.95	2.19	2.08	4.94	<0.012
SD	0.81	0.76	0.75		
Tearful					
Mean	0.21	0.52	0.09	59.91	<0.0005
SD	0.29	0.58	0.17		
Helpless					
Mean	0.23	0.47	0.19	8.99	<0.001
SD	0.42	0.60	0.29		
Happy					
Mean	2.36	2.95	2.51	31.55	<0.0005
SD	0.66	0.66	0.67		
Anxious					
Mean	0.94	1.15	0.75	8.30	<0.001
SD	0.71	0.92	0.69		

Method

Subjects. The subjects were 21 married couples recruited through a local hospital located in a city of 55,000 in the midwestern portion of the United States. All of the subjects were taking instruction in various methods of prepared childbirth. The mean age of the women was 28.4 years (range, 19 to 34) and the mean age of the men was 30.9 years (range, 20 to 46). All subjects were Caucasian, and 10 of the women and five of the men had a college education. Fifteen of the couples had no previous children, and six had one previous child. Nineteen of the husbands were present in the delivery room during the birth of the child.

Procedure. Questionnaires were distributed during the third trimester of pregnancy (prepartum period), during the postpartum period, and then again at 6 months after the birth of the child (follow-up period). In each questionnaire period individual questionnaires were filled out daily by both the mother and father for 10 consecutive days. Questionnaires were administered during 10 days rather than at a single session to minimize the effects of random fluctuations in mood. The subjects were told that the purpose of the study was to gather information about the moods of couples having a baby. All women were in the third trimester of pregnancy when the first set of questionnaires was given to the prospective parents. During the prepartum period a consent form, background information form, and the questionnaires were given to the couples. The couples were instructed to fill out a daily questionnaire for the next 10 consecutive days. They were further told that another set of questionnaires would be given to them as soon as possible after the birth of their child.

The second set of questionnaires was given to the parents within 24 hours of delivery, and in all cases the parents began to fill out the first of the 10 consecutive questionnaires within 2 days after the child's birth. One of the authors (H. W. B.), an obstetrician, evaluated the hospital records of the birth process. All 21 babies weighed at least 5 pounds at birth and any cesarean births or births with severe postpartum medical complications were excluded from this study.

Approximately 6 months after the birth of the child the parents were presented with the final set of questionnaires, and in all cases the parents began to fill out the questionnaires before the baby was 7 months old. The questionnaires that were filled out during the three periods contained the following 20 mood-related adjectives: nervous, lonely, self-confident, irritable, worried, content, depressed, angry, energetic, discouraged, sad, relaxed, miserable, pessimistic, enthusiastic, tearful, helpless, happy, anxious, and upset. The subjects

Table II. Means, SDs, F values, and p values for sex effects

Variable	*Women*	*Men*	*F*	*p*
Lonely				
Mean	0.32	0.16	5.14	<0.035
SD	0.34	0.17		
Irritable				
Mean	0.68	0.48	7.14	<0.015
SD	0.33	0.26		
Depressed				
Mean	0.39	0.21	5.98	<0.024
SD	0.35	0.20		
Angry				
Mean	0.38	0.28	4.76	<0.041
SD	0.36	0.33		
Energetic				
Mean	1.75	2.21	10.93	<0.004
SD	0.47	0.68		
Discouraged				
Mean	0.47	0.29	4.70	<0.042
SD	0.39	0.21		
Sad				
Mean	0.29	0.13	11.75	<0.002
SD	0.22	0.13		
Miserable				
Mean	0.27	0.11	8.70	<0.008
SD	0.24	0.14		
Tearful				
Mean	0.42	0.13	20.91	<0.0005
SD	0.34	0.17		
Upset				
Mean	0.51	0.32	4.64	<0.044
SD	0.38	0.34		

were asked to rate the extent to which each adjective described how they felt that day. The response alternatives were: not at all (scored 0), somewhat (scored 1), moderately (scored 2), very much (scored 3), and extremely (scored 4).

Results

The dependent variables were obtained by averaging the 10 ratings each subject gave for each of the individual moods during prepartum, postpartum, and follow-up periods. Separate 3 (time periods) × 2 (sex) × 21 (couples) analyses of variance were performed on these average scores for each of the 20 moods. Time period and sex were within-subject variables; sex was treated as a within-subject variable because the man and woman of each couple were not independent. Only the effects associated with sex and time period will be reported since differences between individual couples are not relevant to the questions addressed in the present study.

Time period was a significant source of variance for 10 of the 20 moods. At the postpartum period the subjects reported that they experienced more negative affect than they did at either the prepartum or the follow-up period; they were more nervous, less self-confident, more worried, less energetic, more tearful, more helpless, and more anxious. At the same time, however, they reported more positive affect as well; they were less angry, more enthusiastic, and happier. The means, SDs, F values, and p values are presented in Table I.

There were significant sex differences for 10 of the 20 moods. Men reported themselves to be significantly less lonely, irritable, depressed, angry, discouraged, sad, miserable, tearful, and upset than women reported themselves to be. Further, men reported themselves to be significantly more energetic than women. The means, SDs, F values, and p values for these 10 moods are presented in Table II.

There were significant interactions between sex and time period in the analyses of four moods—self-confident, miserable, enthusiastic, and tearful. The means, SDs, F values, and p values for these four interactions are presented in Table III.

Post hoc analysis was performed to further explore the interactions between sex and time period. An 0.05 level of significance was accepted for all post hoc analyses. The significant interaction between sex and time periods for the mood "self-confident" occurred because the ratings of self-confidence by the men did not change across the three periods, while the women rated themselves as significantly more self-confident during the follow-up period than during either of the other two periods.

For the mood "miserable" the interaction between sex and time periods occurred because, while the ratings of the men did not vary across the three periods, those of the women were significantly higher during the postpartum period than during either of the other two periods.

The interaction between sex and time periods was also significant for the mood "enthusiastic." Females reported significantly more enthusiasm during the postpartum and follow-up periods than during the prepartum period while men reported significantly more enthusiasm during the postpartum period than during the other two periods.

Finally, the interaction between sex and time periods was also significant for the mood "tearful." Both the men and the women were significantly more tearful during the postpartum period than during the other two periods. The women were also significantly less tearful during the follow-up period than during the prepartum period.

Comment

The first question that needs to be addressed with the results of the present study is whether the subjects—men or women—experienced postpartum depression. The results of the analyses of variance indicate that there were significant time period effects with 10 of the 20 moods being rated differently during the postpar-

Table III. Means, SDs, F values, p values for interactions between sex and time period

Variable	*Prepartum period*	*Postpartum period*	*Follow-up period*	*F*	*p*
Self-confident					
Women					
Mean	2.22	2.15	2.54	3.28	<0.048
SD	0.69	0.73	0.67		
Men					
Mean	2.45	2.44	2.49		
SD	0.68	0.71	0.69		
Miserable					
Women					
Mean	0.20	0.42	0.18	3.57	<0.037
SD	0.25	0.56	0.27		
Men					
Mean	0.11	0.09	0.11		
SD	0.14	0.23	0.24		
Enthusiastic					
Women					
Mean	1.77	2.00	2.10	4.01	<0.026
SD	0.71	0.77	0.69		
Men					
Mean	2.12	2.38	2.07		
SD	0.88	0.74	0.83		
Tearful					
Women					
Mean	0.35	0.77	0.13	11.92	<0.005
SD	0.35	0.63	0.19		
Men					
Mean	0.07	0.28	0.05		
SD	0.15	0.42	0.14		

tum period than during either the prepartum or the follow-up period. The subjects reported that they felt significantly more nervous, worried, anxious, helpless, tearful, happy, and enthusiastic, as well as significantly less angry, energetic, and self-confident at the postpartum period than during the other two time periods. For all of the moods for which there was a significant time period effect, the postpartum period was the time period that was rated differently from the other two, suggesting that the postpartum period is, emotionally, a somewhat unique period.

Although the results of the present study indicate that the postpartum period is an emotionally unique period, they do not indicate that it is marked by depression. The moods that were rated as being experienced more strongly during the postpartum period were not those typically associated with depression ("depressed," "discouraged," "sad," and "pessimistic"). Rather those rated higher during the postpartum period tended to be moods that were associated either with anxiety and concern with one's ability to cope ("nervous," "worried," "helpless," and "anxious") or with positive emotions such as "enthusiastic," "happy," and "angry" ("angry" decreased during the postpartum period).

In addition to these two main themes, subjects rated themselve as less energetic and more tearful during the postpartum period. It is, of course, not surprising that subjects would rate themselves as having less energy immediately after the birth of a child and during the early days of having a new infant in the home. The fact that "tearful" was rated differently during the postpartum period is more difficult to interpret because there is no direct evidence from the present study as to whether the subjects were feeling tearful for positive, joyous reasons or as a result of more negative feelings. The fact that the subjects reported being more happy, more enthusiastic, and less angry during the postpartum period while they did not report being more depressed, discouraged, sad, pessimistic, or upset, however, suggests that their increased tearfulness may have resulted more from positive affect than from negative affect.

On the basis of the significant time period effects, it appears that the postpartum period is a time of heightened emotions, associated not with depression but rather with anxiety, on the one hand, and increased positive affect, on the other hand. Individuals may be nervous and worried about their ability to cope with the demands of a new infant, but yet, at the same time, they seem to feel very positively about the experience.

The conclusion that the postpartum period was not marked by depression is supported not only by the significant time period effects but also by the absolute levels of depressive affect reported by the subjects. The subjects were asked to rate the extent to which they experienced each of the 20 moods on a scale from 0

(not at all) to 4 (extremely). The mean rating for each of the moods that might be associated with depressive affect (irritable, depressed, discouraged, sad, miserable, pessimistic, and upset) was always less than 1 and usually less than 0.5. This was true even during the postpartum period. It would be difficult to interpret such low ratings of depressive affect as indicating any degree of postpartum depression.

The second question that needs to be addressed is whether the husbands and wives responded in the same way, emotionally, to the postpartum period. There were significant interactions between sex and time period in only four of the analyses of the 20 moods. One of these interactions, the one for "tearful," occurred because the wives exhibited a more extreme change during the postpartum period than their husbands, but the ratings of "tearful" were evaluated in both wives and husbands during the postpartum period. Thus only three of the interactions indicate that wives and husbands responded differently to the postpartum period. First, the interaction between sex and time period for "self-confident" indicated that while the wives felt less self-confident during the postpartum period, their husbands did not. Second, the interaction for "enthusiastic" indicated that while both wives and husbands rated themselves as more enthusiastic during the postpartum period than during the prepartum period, only the husbands indicated a decrease in enthusiasm from the postpartum to the follow-up period. The wives rated themselves as just as enthusiastic during the follow-up period as they did during the postpartum period. Third, the interaction for "miserable" occurred because the wives rated themselves as more miserable during the postpartum period than during the other two periods while the husbands did not rate themselves differently across the three time periods.

The three interactions between sex and time period do not, in our opinion, alter the conclusions drawn on the basis of the main effects for time periods. Both the wives and husbands appeared to experience more anxiety and more positive affect during the postpartum period than during the other two periods. The only interaction that presents any difficuclty for the conclusions that were drawn on the basis of the main effects is that obtained for "miserable." On the basis of the main effects for time period, we concluded that there was no evidence for the postpartum period being associated with depressive affect. The fact that the wives rated themselves as more miserable during the postpartum period suggests that the wives may have experienced some heightened negative affect. However, it seems somewhat unusual that the wives would not also show increases in feelings of "irritable," "depressed," "discouraged," "sad," "pessimistic," and "upset" if they were experiencing depressive affect. It may well be that the wives rated themselves as more miserable during the postpartum period because of physical, rather than psychological, discomfort.

In addition to the time period effects and the interactions between sex and time periods, significant sex differences in mood ratings were also obtained for 10 of the 20 moods. Women rated themselves as more lonely, irritable, depressed, angry, discouraged, sad, miserable, tearful, and upset than men. The men rated themselves as more energetic than the women. These sex differences are difficult to interpret. It is not clear whether women experience a significantly greater degree of negative affect than men or whether women are simply more likely to report negative affect. Perhaps the fathers in our study returned to work and had limited involvement with the child during the postpartum period. The mothers, on the other hand, remained at home with the child and this social isolation could have contributed to the women's negative moods when compared to the husbands' moods.

In conclusion, the results of our study using parents recruited from childbirth classes indicate that the postpartum period is an emotional period for both men and women and that, for the most part, men and women tend to experience the postpartum period in an emotionally similar way. Both husbands and wives tend to rate themselves as more anxious and worried and as experiencing more positive affect. Our sample did not show the high levels of postpartum depression previously reported by others. This could be due to the fact that our participants were taking instruction in childbirth classes.

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Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios

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Amniotic fluid lecithin-to-sphingomyelin ratios are widley used to predict fetal lung maturity. The inclusion of acetone precipitation in the determination of the lecithin/sphingomyelin ratio has been widely accepted as a necessary step for enhancing predictivity. The lecithin/sphingomyelin ratio of 181 amniotic fluid samples was analyzed with and without acetone precipitation. Sensitivity, specificity, and predictive values were compared. Although there was a significant difference in the numeric value ($p < 0.001$) between the two methods, the clinical prediction of the respiratory distress syndrome was not significantly enhanced by acetone precipitation. We conclude that acetone precipitation fails to improve the prediction of fetal lung maturity by lecithin/sphingomyelin ratio analysis. (AM J OBSTET GYNECOL 1986;154:1023-6.)

Key words: Acetone precipitation, lecithin/sphingomyelin ratio

Despite considerable controversy in the past, acetone precipitation before the measurement of amniotic fluid lecithin is still widely accepted as being indispensable. The rationale for the use of acetone precipitation has been that total amniotic fluid lecithin is not truly representative of "surface active" lecithin and that acetone precipitation is capable of separating the surface-active lecithin (disaturated lecithin) from the other lecithin components.[1] However, it has been shown that acetone precipitated extracts of amniotic fluid are not exclusively composed of disaturated lecithin.[2] Furthermore, the original study by Gluck et al.[3] suggesting acetone precipitation as necessary for the isolation of saturated lecithin ironically used a detection method that measured only unsaturated lecithin.[4]

Gluck et al.[3, 5] first reported that the respiratory distress syndrome in newborn infants could be predicted from measurements of the lecithin/sphingomyelin (L/S) ratio in amniotic fluid. With the inclusion of the acetone precipitation step in his procedure, Gluck et al.[6] reported an L/S ratio beyond 2.0 was associated with little or no risk for respiratory distress syndrome. However, the inclusion of the acetone precipitation step in determining the L/S ratio of amniotic fluid has never been shown by parallel testing of the same sample to enhance predicitivity. We therefore undertook a prospective study to determine the effect of acetone precipitation on the clinical prediction of respiratory distress syndrome.

Material and methods

Specimen collection. One hundred eighty-one amniotic fluid specimens collected from separate pregnancies of varying gestational ages were analyzed for phospholipid composition. The samples were analyzed regardless of the source of amniotic fluid (amniocentesis versus vaginal pool) or complications of pregnancy (Table I). Samples with trace meconium or blood contamination were included.

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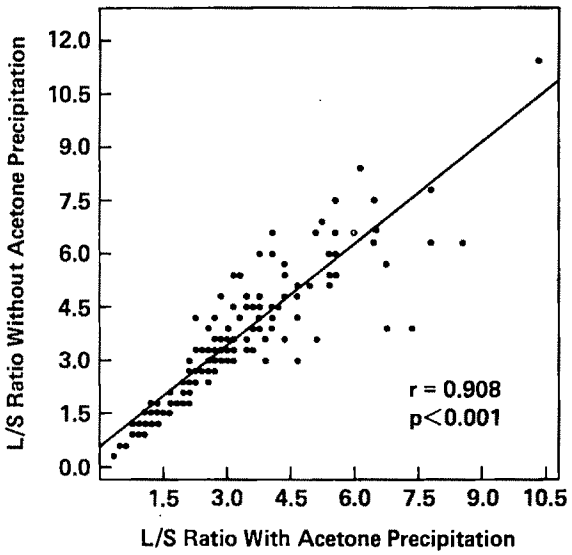


Fig. 1. Comparison of the L/S value without acetone precipitation to the same specimen when acetone precipitation was used.

Table I. Status of pregnancy in patients undergoing amniotic fluid analysis

Pregnancies	*No.*
Uncomplicated	100
Complicated by	
Rupture of the membranes >24 hr	15
Diabetes mellitus	
Class A	17
Class B	20
Class C, D, R	3
Hypertensive disease	19
Erythroblastosis fetalis	2
Abnormal placentation	5
Total	181

Specimen storage. Amniotic fluid specimens were immediately centrifuged on receipt at 1000 × g for 10 minutes and the supernatant decanted. If the determination of phospholipids was going to be done within 4 hours, the specimen was refrigerated. Otherwise, the centrifuged specimen was stored at −20° C until analyzed. All specimens were analyzed within 48 hours of being received.

Extraction. The supernatant was mixed with an equal volume of methanol before the addition of a third volume of chloroform. The phases were mixed vigorously for a few seconds and then centrifuged at 1000 × g for 10 minutes to separate the layers. The chloroform layer was decanted and evaporated to dryness at 37° C under a stream of air. The dried extract from 1 ml of amniotic fluid then underwent the acetone precipitation procedure while a duplicate aliquot of the same extract was reconstituted in chloroform for thin-layer chromatography.

Table II. Comparison of the ability to predict respiratory distress syndrome (RDS) by L/S ratio with and without acetone precipitation*

Test	*RDS*	*Nc RDS*	*Total*
L/S without acetone precipitation			
L/S ratio < 2.0	15	7	22
L/S ratio ≥ 2.0	2	150	152
L/S with acetone precipitation			
Acetone < 2.0	15	10	25
Acetone ≥ 2.0	2	147	149

*All samples were obtained ≤72 hours before delivery.

Acetone precipitation. One hundred microliters of cold, anhydrous acetone was added to the tube of dried extract, which had been placed in an ice bath for 2 minutes and the tube held on ice for an additional 5 minutes. The tube was then inverted, the acetone allowed to drain off, and a second 100 μl of cold anhydrous acetone added as a rinse and drained off immediately. Any residual acetone was dried with an air stream. The extract was reconstituted in chloroform and loaded onto the thin-layer chromatography lane next to the acetone-precipitated extract.

Chromatography. Extracts and standards in chloroform were streaked onto thin-layer chromatography plates (catalog No. 4865-82, Whatman, Inc., Clifton, New Jersey). After evaporation of the loading solvent at room temperature, the plates were allowed to develop for 70 minutes in tanks containing the following solvent mixture: 30 ml of chloroform, 25 ml of isopropanol, 25 ml of triethylamine, 9 ml of methanol, and 7 ml of water.[7] After development the plates were briefly air dried and heated to 160° C for 5 minutes before a cupric acetate reagent was applied.[8] Bands were identified by migration relative to known standard compounds. Both acetone-precipitated and non–acetone-precipitated extracts of the same amniotic fluid specimen were chromatographed on adjacent lanes of the same plate. Band intensities were determined by transmission densitometry. The densitometric ratio required for the heating standard (catalog No. LS30, Sigma Chemical, St. Louis) was 1.65 ± 0.20. Band development was at 160° C.

Clinical evaluation of infant outcome. Each infant's outcome was evaluated by two of the investigators (D. B. C. and T. S.), independent of the results obtained from amniotic fluid analysis. The following signs and symptoms were considered diagnostic of the respiratory distress syndrome: grunting, tachypnea, retraction, nasal flaring, cyanosis on room air, and a reticulogranular ground glass appearance with air bronchograms on chest x-ray examination. The necessity for oxygen therapy for a minimum of 24 hours was re-

Table III. Value of the different methods of L/S ratio determination in the prediction of respiratory distress syndrome (RDS)

Test	No. of infants examined	Sensitivity*		Specificity†		Predictive value of abnormal tests‡		Predictive value of normal tests§		Efficiency‖	
		n	%	n	%	n	%	n	%	n	%
L/S without acetone precipitation	175	15/17	88	150/157	95	15/22	68	150/152	99	165/174	95
L/S with acetone precipitation	175	15/17	88	147/157	94	15/25	60	147/149	99	162/174	93

The definitions given below which are used to establish the value of a diagnostic test are from reference 17.
*Number of L/S ratios <2.0 ÷ by number of infants with RDS.
†Number of L/S ratios ≥2.0 ÷ by number of infants without RDS.
‡Number of infants with RDS ÷ by number of infants with an L/S ratio <2.0.
§Number of infants without RDS ÷ by number with L/S ratios to ≥2.0.
‖Number of true positive tests + the number of true negative tests ÷ by total number of tests.

quired. The diagnosis of mild respiratory distress syndrome was made when an oxygen-hood was required to maintain oxygenation, moderate respiratory distress syndrome when continuous positive airway pressure was required, and severe respiratory distress syndrome when endotracheal intubation was required.

In cases for which the prediction of respiratory distress syndrome was tested, all infants were born within 72 hours of the amniotic fluid collection.

Results

We first compared the numeric result of the L/S ratio determined by the exclusion of the acetone precipitation step (nonprecipitated L/S) to the L/S ratio determined by the inclusion of the acetone precipitation step. With use of the paired Student's *t* test, there was a significant difference in the L/S ratio values obtained between the two methods ($t = 6.0$, $p < 0.001$). Calculation of the least-squares linear regression line showed that a nonprecipitated L/S of 2.0 was equivalent to an L/S with acetone precipitation of 1.9 (acetone-precipitated L/S ratio = 0.88 (L/S nonprecipitated) + 0.1; see Fig. 1).

Next we compared the clinical predictivity for respiratory distress syndrome of the nonprecipitated L/S values with the acetone-precipitated L/S ratios (Table II). There were 175 cases in which delivery occurred within 72 hours, 17 involving infants in whom respiratory distress syndrome developed (five cases were mild, four were moderate, and eight were severe). Prediction of respiratory distress syndrome on the basis of the nonprecipitated L/S ratio resulted in two false negative tests out of 152 mature tests (1.3%) and seven false positive tests out of 22 immature tests (31.8%). Prediction on the basis of the acetone-precipitated L/S ratio resulted in two false negative tests out of 149 mature values (1.3%) and 10 false positive tests out of 25 immature values (40.0%).

Table III shows the value of the two methods as assessed by multiple parameters. The sensitivity, specificity, and predictive values for a positive and negative test were not appreciably different.

Comment

In 1967 Gluck et al.[1] measured the surface activity of acetone-precipitated and acetone-soluble lecithins. They showed that toward the end of gestation, the acetone-precipitated fraction exhibited "normal" surface activity, whereas the total lecithin and acetone soluble fraction did not. Subsequently it was shown that the percentage of dipalmitoyl lecithin was greater in the acetone precipitate than in the acetone supernatant.[9] It was concluded from these studies that the surface-active component was dipalmitoyl lecithin and that it was separated from the total lecithin fraction by acetone precipitation.

This concept has resulted in the historic emphasis that the measurement of saturated phosphatidylcholine provides the most accurate method for predicting respiratory distress syndrome. However, many of the detection techniques measuring only unsaturated lecithin have proved reliable.[5, 10-12] The ability to predict fetal pulmonary maturation from unsaturated lecithin is possible because like saturated lecithin, it increases with gestational age.[3, 13] Therefore the removal of unsaturated lecithin by acetone precipitation appears to be an unnecessary goal.

The solubility of lecithin in cold acetone as reviewed by Brown and Duck-Chong[14] is reported to be dependent on numerous factors including (1) the length of the fatty acid side chains and/or their degree of saturation, (2) the presence of other phospholipids, (3) the

concentration of phospholipids, (4) the time allowed for precipitation, (5) the temperature during precipitation and centrifugation, (6) the water content of acetone, and (7) the use of chloroform to moisten the dried lipid extract before the addition of acetone. Since ≥80% of sphingomyelin is precipitated by cold acetone whereas 60% to 90% of lecithin is precipitated,[15, 16] acetone precipitation should cause a decrease in the L/S ratio. These factors decrease precision of the test, complicate comparison of results between laboratories, and add additional performance time.

The results of this clinical study clearly demonstrate that the inclusion of acetone precipitation in the determination of the L/S ratio does not enhance the predictivity of the test for detection of the respiratory distress syndrome.

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Serum fructosamine: A screening test for diabetes in pregnancy

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Serum fructosamine levels were measured in 1200 pregnant women at different stages of gestation, 167 of them having a glucose tolerance test. There was a significant fall in fructosamine levels throughout pregnancy and details of normal values at different gestational stages are presented. A good correlation was found between fructosamine and glucose tolerance test ($r = 0.813$). Nine women had gestational diabetes diagnosed by glucose tolerance test and eight of those had a serum fructosamine level greater than the ninety-fifth percentile. This study confirms our previous findings that serum fructosamine may be a useful screening test for gestational diabetes. (Am J Obstet Gynecol 1986;154:1027-30.)

Key words: Fructosamine, diabetic pregnancy, screening test

Diagnosis of diabetes in pregnancy is difficult and has traditionally depended on an assessment of clinical risk with oral glucose tolerance testing in suspected cases. Results are unsatisfactory with detection of only 62% of cases at best.[1] Systematic screening by blood glucose measurement 1 hour after an oral glucose load detects 79% of diabetic pregnancies but is inconvenient and time-consuming for the patient and provides a high (13%) rate of false positive observations.[2]

We have recently shown that 85% of women with diabetes in pregnancy have higher levels of serum fructosamine than nondiabetic pregnant women, suggesting that fructosamine might be a useful screening test for gestational diabetes.[3] Fructosamine concentrations reflect blood glucose control during the previous 1 to 3 weeks[4] but do not change significantly from day to day, offering the convenience of random blood sampling.[5] Major practical advantages of the test include technical simplicity, automation, and reliable standards.[6]

This study was designed to investigate the association between fructosamine levels and gestational age and to assess the usefulness of fructosamine as a screening test in the clinical situation.

Patients and methods

Patients. The nonpregnant reference interval for serum fructosamine was determined in 3664 female volunteers aged <1 to 89 (median 23) years who participated in a community health survey. Fructosamine concentrations during pregnancy were measured in 1200 women aged 13 to 44 (median 23) years attending the antenatal clinic at National Women's Hospital, Auckland, New Zealand. Serum samples were collected for fructosamine analysis in conjunction with normal antenatal screening tests at booking and 28 and 36 weeks' gestation. Results were not reported to the clinical team. No women with known diabetes or previous gestational diabetes were included in this reference population.

One hundred sixty-seven pregnant patients selected on clinical grounds had a 100 gm 3-hour glucose tolerance test ordered by the clinician in charge. Plasma

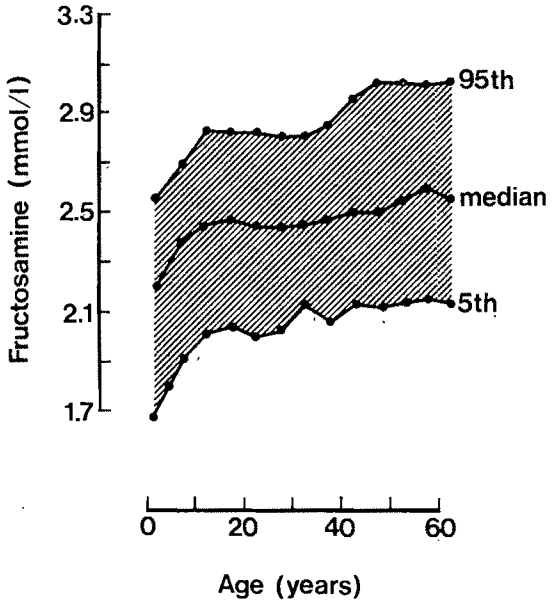


Fig. 1. Relationship between serum fructosamine and age in 3664 nonpregnant women. *Hatched area* is the reference interval.

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Table I. Fructosamine measurements in 1200 pregnant women at different gestational stages compared with concentrations in 1650 nonpregnant women

	No. of observations	*Median (mmol/L)*	*5th Percentile (mmol/L)*	*95th Percentile (mmol/L)*
Nonpregnant women	1650	2.46	2.02	2.82
Pregnant women (weeks' gestation)				
4-8	90	2.46	2.21	2.67
8-12	254	2.47	2.23	2.68
12-16	167	2.46	2.19	2.70
16-20	138	2.43	2.19	2.62
20-24	130	2.39	2.14	2.61
24-28	189	2.35	2.15	2.55
28-32	215	2.36	2.15	2.61
32-36	152	2.31	2.11	2.58
36-40	232	2.31	2.11	2.58

Table II. Clinical particulars of nine gestational diabetic patients

				Glucose tolerance test						
Patient	*Age (yr)*	*Maternal weight (kg)*	*Birth-weight (gm)*	*Fasting* (mg/dl)*	*1 hr (mg/dl)*	*2 hr (mg/dl)*	*3 hr (mg/dl)*	*Weeks' gestation*	*Fructosamine† (mmol/l)*	*Weeks' gestation*
1	35	91	2840	234	448	369	299	12	3.70	11
2	43	112	3940	191	308	306	324	13	2.88	14
3	37	84	3460	151	329	286	202	13	2.74	16
4	35	76	4150	105	207	176	149	25	2.41	18
									2.72	28
5	28	57	3070	99	216	220	202	30	2.48	18
									2.80	30
6	34	95	4560	121	225	229	148	31	2.66	23
									2.71	37
7	32	104	3960	140	230	243	178	35	2.72	29
									2.74	39
8	23	90	3900	137	229	227	223	35	2.75	36
9	26	107	4440	112	238	191	101	37	2.85	20
									2.88	39

For conversion of glucose values from traditional to SI units: 1 mg/dl = 0.056 mmol/L.

*Diabetic fasting plasma glucose is >105 mg/dl (5.8 mmol/L).

†Nondiabetic fructosamine values at different gestational stages are shown in Table I.

glucose results were interpreted by means of O'Sullivan's criteria.[7]

Analytical techniques. Serum fructosamine concentrations[6] were determined with commercial reagents and an automated centrifugal analyzer (F. Hoffman-La Roche, Basel, Switzerland). Changes in the calibration of the fructosamine assay[6] have increased results by about 0.8 mmol/L compared with previously published values.[3-5] Plasma glucose was measured with a glucose oxidase electrode (Beckman Instruments, Fullerton, California). The between-batch imprecisions for fructosamine and glucose were 1.4% and 2.7%, respectively.

Statistical methods. We compared fructosamine concentrations at different gestational ages with the use of Tukey's multiple range test for comparison of intervals.[8] Statistical significance was determined by Student's *t* test for paired or unpaired data as appropriate.

Results

Nondiabetic values. The range of fructosamine results in 3664 nonpregnant female volunteers are presented in Fig. 1. There were no significant age-related changes ($p > 0.05$) during the second, third, and fourth decades. Fructosamine concentrations for the 1650 women aged 15 to 40 (median 26) years were therefore used to derive the nonpregnant reference interval (Table I). There was no significant difference ($p > 0.05$) between nonpregnant and pregnant values during the first trimester of pregnancy [mean first-trimester fructosamine = 2.46 ± 0.16 (SD) mmol/L]. However, significant differences ($p < 0.05$) were noted both between first- and second-trimester [mean second trimester fructosamine = 2.41 ± 0.14 (SD) mmol/L] and between second- and third-trimester [mean third-trimester fructosamine = 2.34 ± 0.14 (SD) mmol/L] results (Fig. 2). Fructosamine concentrations at different gestational stages are presented in Table I.

Table III. Correlation between maternal glucose tolerance test results and serum fructosamine concentration in 167 pregnant women

Observations (n)	*Time between tests (wk)*	*Correlation coefficient*	
		Fasting glucose	*Area under curve*
30	<2	0.81	0.77
45	<3	0.79	0.74
63	<4	0.75	0.70
85	<5	0.64	0.60
111	<6	0.54	0.45
123	<7	0.54	0.43
139	<8	0.48	0.34
167	8+	0.42	0.24

All significant at $p < 0.001$

Comparison with glucose tolerance test. Diabetes was confirmed in nine of the 167 pregnant women who underwent a glucose tolerance test (prevalence 5.4%). Fructosamine measurements exceeded the ninety-fifth percentile for gestational age in eight of the nine individuals (Table II). A highly significant association ($p < 0.001$) was found between fructosamine levels and both fasting plasma glucose and area under the glucose tolerance curve (Table III). This association improved with decreasing time between fructosamine measurement and the glucose tolerance test.

Performance of fructosamine as a screening test. Predictive value of fructosamine screening tests are influenced both by the time between screening and positive glucose tolerance test and by the prevalence of diabetes in different subgroups. Nevertheless, our results suggest an optimal interval of 4 weeks between tests with six true positive, one false negative, and three false positive observations in the 63 women whose fructosamine level was measured during the 4 weeks preceding or after glucose tolerance testing (Table IV). Sensitivity, specificity, predictive value positive, and predictive value negative calculated for these 63 patients were 86%, 95%, 66%, and 98%, respectively.

Comment

Compared with nonpregnant values, maternal fructosamine concentration begins to fall in the second trimester of pregnancy and remains reduced until the end of pregnancy (Table I). Most of the change is presumably due to the fall in fasting or basal glucose concentrations, which occurs early in the first trimester.[9] Serum total protein and albumin concentrations also fall after the first 3 months of pregnancy[10] and may contribute to this 6.1% reduction of fructosamine level. Nevertheless, a group-specific reference range is required since correction for individual serum total protein and albumin concentrations is not beneficial.[5]

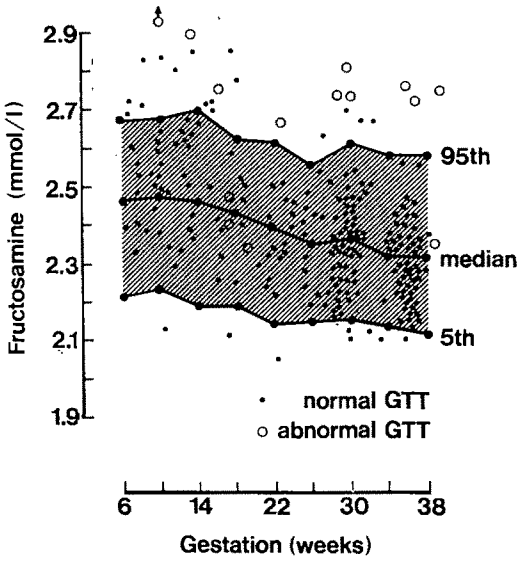


Fig. 2. Relationship between serum fructosamine and gestational age in 1200 pregnant women. *Hatched area* is the reference interval. Results from 167 patients who had oral glucose tolerance testing are superimposed.

Glucose intolerance may develop at any stage of pregnancy and must necessarily precede an elevation of serum fructosamine concentration. Fructosamines are formed by the nonenzymatic glycosylation of serum proteins.[6] The Amadori rearrangement reaction is rate-limiting and imposes a delay before changes in glycemia are reflected in the fructosamine result.[11] This concept is supported by our findings in the current study. We observed a highly significant correlation between maternal fructosamine and both fasting plasma glucose ($r = 0.81$) and area under the curve ($r = 0.76$) that was maximal after a lag period of 2 weeks (Table III).

Clinical application of fructosamine as a screening test for diabetes in pregnancy therefore requires discrimination values that are both adjusted for gestational age and designed to compensate for the rate of glycosylation of serum proteins. We chose the ninety-fifth percentile as our discrimination value but expect a false positive rate of <5% since the incidence of gestational diabetes in our clinic population is probably 4% to 5%.[12]

Overall sensitivity (86%) and specificity (95%) of the test compared favorably with those of other diabetic screening procedures[1,2] and detected eight of the nine new cases of diabetes (Table II). However, glucose tolerance tests in this study were only performed on a "high-risk" subgroup with a higher prevalence of gestational diabetes than in the general population. Because the positive and negative predictive values of screening tests are influenced by the prevalence of diabetes in the population, these figures may overestimate the performance of the fructosamine assay.

The cases of gestational diabetes in the current study

Table IV. Predictive value of fructosamine screening tests for gestational diabetes

n	*Time between GTT and fructosamine (wk)*	*Prevalence**	*Sensitivity*	*Specificity*	*Predictive value*	
					Positive	*Negative*
30	<2	16.6	0.80	0.92	0.66	0.96
63	<4	11.1	0.86	0.95	0.66	0.98
139	<8	6.5	0.89	0.88	0.35	0.99
167	8+	5.4	0.89	0.90	0.35	0.99

Diagnosis of gestational diabetes, based on a 100 gm oral glucose tolerance test (GTT) interpreted according to O'Sullivan's criteria.[7]

*Refers to the number of gestational diabetic patients in each subgroup.

were also relatively severe, with seven of the nine patients having fasting glucose levels >105 mg/dl (5.8 mmol/L). Indeed, six of the nine individuals had fasting plasma glucose levels >120 mg/dl, the threshold for diagnosing diabetes in a nonpregnant individual. This study does not determine whether fructosamine is sensitive enough to detect gestational diabetes with less severe glucose intolerance and particularly those cases where patients have normal fasting plasma glucose concentrations. Nevertheless, fructosamine would have detected 84 (90%) of the 93 cases of gestational diabetes diagnosed in this unit during the past 3 years.

The single patient with false negative fructosamine results (Patient 9, Table II) had definite gestational diabetes with a fasting plasma glucose level of 112 mg/dl (6.2 mmol/L) at 37 weeks and was delivered of a macrosomic infant (birth weight 4440 gm). We have observed similar false negative fructosamine results in a number of other women with gross maternal obesity and suspect interference in the assay from maternal hypertriglyceridemia.[6] Inspection of the sample before analysis for gross lipemia could prevent this problem.

In our initial report we advocated fructosamine measurement at 28 to 30 weeks' gestation.[3] Since case detection is improved by repeated sampling throughout pregnancy (Table II) at little extra cost or inconvenience to the patient, we now recommend routine serum fructosamine measurement at intervals of 4 to 6 weeks during pregnancy. Our results suggest that fructosamine estimation may be a simple and effective screening test for gestational diabetes.

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Should the fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state?

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To determine whether the 50 gm, 1-hour plasma glucose screening test for gestational diabetes should be administered in the fasted or fed state, 50 presumed normal and 20 gestational diabetic pregnant women in the early third trimester underwent this test twice, once under each condition, within a 1-week interval. There was no difference in test results under the two conditions among the normal individuals (fasted 118.4 ± 24.7 mg/dl; fed 115.8 ± 23.4 mg/dl). However, when the test was administered to women with known gestational diabetes, the result was significantly ($p = 0.011$) higher if patients were fasted (173.9 ± 28.8 mg/dl) than if they had been given a standard 600 kcal meal 1 hour previously (154.8 ± 24.1 mg/dl). The effect of these two conditions on the sensitivity and specificity of the screening test is described, and it is suggested that the threshold for glucose tolerance testing be 130 mg/dl if the test is administered in the fed state. (Am J Obstet Gynecol 1986;154:1031-5.)

Key words: Gestational diabetes, diabetes screening test, diabetes and pregnancy; glucose tolerance test

The Second International Workshop-Conference on Gestational Diabetes Mellitus* has recommended that "*all* pregnant women should be screened for glucose intolerance since selective screening based on clinical attributes or past obstetric history has been shown to be inadequate. Screening should be by glucose measurement in blood." It was recommended that a 50 gm oral glucose screening test be performed at 24 to 28 weeks of pregnancy, ". . . given without regard to time of the last meal or the time of day."[1] When this 50 gm glucose screening test was described by O'Sullivan et al.,[2] it was similarly given without stipulation as to fasting or fed state.

In the report of O'Sullivan et al.[2] on the 50 gm screening test, glucose was measured in whole blood by the Somogyi-Nelson technique. A threshold of 130 mg/dl yielded a sensitivity of 79% and specificity of 87% for detecting gestational diabetes. With the change from whole blood to plasma measurements in most laboratories, and with current more specific methods of glucose measurement, varying recommendations for the appropriate threshold have been made, ranging from 135 mg/dl[3] to 150 mg/dl.[4] Our group has published data suggesting that a threshold of 135 mg/dl would be more sensitive, with use of data collected on individuals who took the test in the fasting state.[3] It seems reasonable to predict that the fasting individual might respond to a glucose load in a quantitatively different manner than would an individual in the fed state. The induction of a state of accelerated starvation by pregnancy, with insulin resistance, has been documented.[5,6] After a mixed meal, insulin and glucagon are secreted, and these hormones might alter the response to a subsequent glucose challenge. It might be expected that the previous ingestion of a mixed meal would blunt the response to a glucose challenge. On the other hand, after a meal is ingested, circulating glucose increases, so that a glucose load superimposed on this already increased plasma glucose level might result in a higher screening test value compared to that obtained in the fasting state.

The present study was designed to test the hypothesis that the ingestion of a standard mixed nutrient meal 1 hour before testing, as compared to the fasting state, would significantly change the response to the 50 gm

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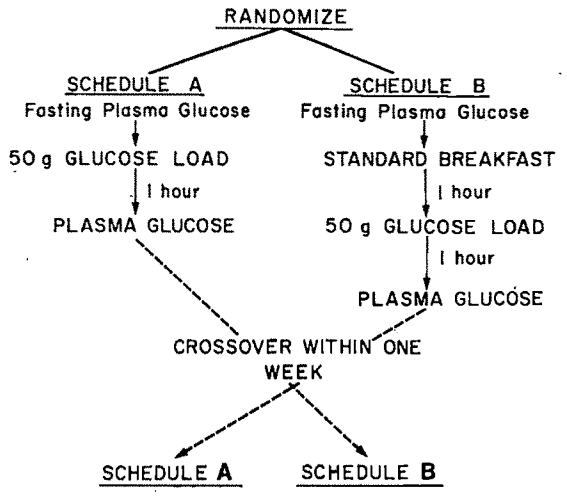


Fig. 1. Randomized crossover design for the two testing conditions.

Table I. Demographic data

	Presumed normal subjects (n = 50)	*Known gestational diabetic patients (n = 20)*
Age (yr)*	27.4 ± 3.4	30.0 ± 5.0
Gestational age of study group with meal (wk)	26.9 ± 1.3	30.4 ± 2.2
Gestational age of study group with no meal (wk)	27.0 ± 1.2	30.5 ± 2.0
Obesity (%)†	4/50 (8)	4/20 (20)
Other risk factors‡	18/50 (36)	11/20 (55)
No risk factors	29/50 (58)	8/20 (40)

*Mean ± SD.

†Above eighty-fifth percentile of relative weight for height and age (National Center for Health Statistics, 1983).

‡Family history of diabetes, previous macrosomic baby (>9 pounds), poor obstetric history.

oral glucose screening test in normal pregnant individuals and in women with gestational diabetes.

Material and methods

The subjects of this study included 50 presumed normal pregnant women about to undergo screening for gestational diabetes and an additional 20 women already diagnosed as having gestational diabetes by the criteria of O'Sullivan and Mahan,[7] as modified by Carpenter and Coustan.[8] Both groups of subjects were recruited from private obstetricians' offices and clinics, affiliated with the Women and Infants Hospital of Rhode Island. The subjects were between 25 and 33 weeks of gestation at the time of study. Women with gestational diabetes had not yet started on their dietary regimen. No patient was receiving insulin, either therapeutically or prophylactically, at the time of study. Demographic data describing these 70 individuals are presented in Table I.

The experimental protocol was approved by the Research and Human Subjects Committee of the Women and Infants Hospital of Rhode Island. All subjects fasted overnight before reporting for study. Twenty-four hour dietary recall forms revealed that all subjects had ingested a minimum of 200 gm of carbohydrate on the day before the study. By means of a table of random numbers, the subjects were assigned one of two test schedules (Fig. 1). Subjects assigned to schedule A underwent the 50 gm glucose screening test in the fasting state, whereas those in schedule B did so in the fed state. For schedule A a fasting plasma glucose sample was first drawn, and then subjects drank a 50 gm oral glucose load (Limeondex, Curtin Matheson Scientific, Inc., Houston, Texas) over 5 minutes. Plasma glucose was again measured 1 hour later. For schedule B the subjects also had a fasting plasma glucose sample drawn but then consumed a standard mixed breakfast, consisting of approximately 600 kcal made up of approximately 52 gm of carbohydrate, 28 gm of protein, and 31 gm of fat. One hour after completion of the mixed meal, a 50 gm oral glucose load was ingested. A plasma glucose level was obtained 1 hour after ingestion of the glucose load. Within 1 week each subject was retested under the opposite conditions of her original test. The 50 presumed normal subjects and the 20 with gestational diabetes all completed the same protocol but were considered as two separate groups for purposes of randomization.

Blood samples were collected on ice in heparinized tubes, and centrifuged within 1 hour. Plasma glucose was determined with use of the glucose oxidase method (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Presumed normal subjects were evaluated with a 100 gm, 3-hour oral glucose tolerance test if either screening test value exceeded or equaled 130 mg/dl. The criteria of O'Sullivan and Mahan,[7] as modified for plasma and glucose oxidase,[8] were used as the diagnostic standard for gestational diabetes. All subjects with known gestational diabetes had previously been diagnosed with such a test, and oral glucose tolerance tests were not repeated in this group.

Test results under the two conditions were compared with use of the *t* test for paired samples. Differences in screening test function under fasting and fed conditions were evaluated with use of Fisher's exact test.

Results

Table II depicts the mean plasma glucose results for the 50 presumed normal individuals who were screened. There was no significant difference between the fasting plasma glucose and 50 gm, 1-hour screening test values under the fasted and fed conditions. Among the 50 subjects there were four who met the criteria for gestational diabetes. Two had abnormal screening

Table II. Results of fasting and 50 gm, 1-hour screening tests in 50 presumed normal subjects

	Plasma glucose in 50 presumed normal subjects (n = 50) (mg/dl)		
	No-meal day (schedule A)	*Meal day (schedule B)*	*p*
Fasting	82.7 ± 7.6	82.1 ± 7.9	0.436
1 hour after 50 gm load	118.4 ± 24.7	115.8 ± 23.4	0.422

*Values expressed as mean ± SD.

Table III. Results of fasting and 50 gm, 1-hour screening tests in 20 previously diagnosed gestational diabetic subjects

	Plasma glucose in 20 gestational diabetic subjects (mg/dl)		
	No-meal day (schedule A)	*Meal day (schedule B)*	*p*
Fasting	95.3 ± 12.4	94.2 ± 10.2	0.628
1 hour after 50 gm load	173.9 ± 28.8	154.8 ± 24.1	0.011

Values expressed as mean ± SD.

tests in the fasted (160 and 146 mg/dl) but not the fed (119 and 118 mg/dl) state. Another had an abnormal result in the fed (132 mg/dl) but not the fasted (113 mg/dl) state. The fourth had an abnormal screening test under both conditions (158 and 167 mg/dl).

Table III depicts the plasma glucose values for the 20 subjects with known gestational diabetes. There was no difference in fasting values on the two testing days. However, there was a significant increase in the 50 gm, 1-hour screening test value when the test was administered in the fasted state, as compared to the fed state.

Table IV depicts the sensitivity of the 50 gm, 1-hour screening test when different thresholds were used and when the test was administered in either the fasted or fed state. The four subjects diagnosed as having gestational diabetes from among the 50 presumed normal individuals were added to the 20 known gestational diabetic patients for these calculations. The screening test was significantly more sensitive, at thresholds of 145 and 150 mg/dl, when administered in the fasted as compared to the fed state.

Table V depicts the specificity of the test in detecting normal individuals when different thresholds are used and when the test is administered in either the fasted or the fed state. There was no significant difference in specificity between the two conditions at any threshold evaluated.

To express the degree of glucose tolerance test abnormality in individual subjects, the sum of the amount

Table IV. Effect of fasted and fed states on test sensitivity (number of correctly identified gestational diabetic patients divided by total number of gestational diabetic patients in the population) at various test thresholds

Threshold used (mg/dl)	*Test sensitivity of gestational diabetic subjects*				
	Fasting		*Fed*		
	n	%	*n*	%	*p**
130	23/24	96	20/24	83	0.174
135	22/24	92	17/24	71	0.068
140	22/24	92	17/24	71	0.068
145	21/24	88	14/24	58	0.024
150	20/24	83	11/24	46	0.007

*Fisher's exact test.

Table V. Effect of fasted and fed states on test specificity (number of correctly identified normal individuals divided by total number of normal individuals in the population) at various test thresholds

Threshold used (mg/dl)	*Test specificity of nondiabetics*				
	Fasted		*Fed*		
	n	%	*n*	%	*p**
130	24/46	52	21/46	46	0.500
135	26/46	56	32/46	70	0.140
140	40/46	87	36/46	78	0.085
145	41/46	89	40/46	87	0.500
150	44/46	96	44/46	96	—

*Fisher's exact test.

by which the two most abnormal values exceed the upper limits of normal[8] was calculated (ΔGTT). The mean ΔGTT for the 20 known gestational diabetic patients in this study was 51.4 (±47.0) mg/dl. The four subjects who were not detected under fed conditions at a threshold of 135 mg/dl had Δ scores of 15, 21, 20, and 26 mg/dl. The single subject who would have been missed with use of the same threshold under fasting conditions had a Δ score of 11 mg/dl. Thus these individuals had mildly abnormal glucose tolerance tests.

Comment

The function of a screening test is to provide a convenient, reliable, inexpensive, and safe means to choose individuals for further testing to detect a given disease state, in this case gestational diabetes. The 50 gm oral diabetes screening test has been shown to be inexpensive and reliable when compared to full 3-hour glucose tolerance testing of all patients meeting historic risk criteria.[2] Administration of the 50 gm glucose load in the fed state adds to the convenience of the test, ren-

dering unnecessary the scheduling in advance of the procedure and allowing immediate testing when the patient appears at an advanced state of pregnancy. No dietary preparation is required, which adds to patient acceptance. However, the results of the present study suggest that when the test is to be given in the fed state, a low enough threshold should be used to avoid missing a significant proportion of the individuals with gestational diabetes in the population. For example, if the test is administered 1 hour after a meal, a 140 mg/dl threshold will detect only 71% of patients with gestational diabetes and a 150 mg/dl threshold will detect only 46%. If the test is administered to fasting patients, however, 92% of gestational diabetic patients will be detected when the threshold is 140 mg/dl, and 83% when it is 150 mg/dl. These results are similar to those we have previously reported in which a threshold of 143 mg/dl yielded a sensitivity of 83% when the test was administered in the fasting state.[3]

It should be made clear that, given our study design, sensitivity calculations may overestimate the true sensitivity of the tests, since only those patients with plasma glucose levels >130 mg/dl underwent a 100 gm, 3-hour oral glucose tolerance test. Thus, if there are individuals within the population whose screening test values were <130 mg/dl, but who would have had an abnormal 3-hour oral glucose tolerance test had this been performed, they would be missed. Since most current recommendations are for the use of thresholds of >130 mg/dl, this fact only adds emphasis to the argument for a threshold of <140 to 150 mg/dl.

Because a standard mixed meal was used, the specific sensitivity and specificity calculations may not be applicable to glucose screening tests administered after the ingestion of unknown quantities and qualities of nutrients. Similarly, because a standard 1-hour interval was employed between the meal and the administration of the 50 gm glucose load, other intervals might yield differing results. The 1-hour time period was attractive because it was estimated that in the usual clinical situation, pregnant women eating a meal at home would be unlikely to appear for screening less than 1 hour later.

The 1-hour time period was also chosen because it was anticipated that the alteration in response to the glucose challenge would be greatest at this time (as compared to two or more hours). However, in a study of six nonpregnant type II diabetic individuals,[9] when 50 gm of glucose were ingested alone or in combination with 50 gm of protein, the plasma glucose level 1 hour later was the same under both conditions, but at over 4 hours the plasma glucose response was significantly lower when protein had been ingested than when the glucose was taken alone. Thus the direction of the effect reported herein is likely to be similar with other time intervals and other types of mixed meals. We hypothesize that the screening test value was lower when the test was administered in the fed state as compared to the fasting state because the previous ingestion of a mixed meal dampened the usual state of insulin resistance observed in fasting pregnant women.

Cheney et al.[10] have suggested that obese gestational diabetic patients manifest different degrees of glucose and insulin response to a mixed meal than do nonobese individuals. Our patient group contained only four obese gestational diabetic individuals, and their plasma glucose response to the glucose challenge under the two testing conditions did not differ qualitatively or quantitatively from those of the nonobese women with gestational diabetes, but no comment can be made about their response to the mixed meal itself.

Sensitivity and specificity can be calculated from the present study, since these test functions do not depend greatly on the prevalence of the disease in the population tested. However, it would be inappropriate to calculate the positive and negative predictive accuracy from our data, since these functions are largely dependent on disease prevalence. Our subjects are not a truly population-based sample, because the 50 women were recruited on the basis of their willingness to participate in the study. Since the study design was intended to test the hypothesis that the fasting or fed state of the individual has an impact on the results of the diabetes screening test and since each subject was used as her own control, the possibility of selection bias does not prejudice the results reported. This possibility does, however, preclude interpretation of the data in areas where prevalence is a critical factor.

Finally, it should be pointed out that data pertaining to the reproducibility of the 50 gm, 1-hour screening test are not available at present. A study in which the same subjects had the test performed under the same conditions on two or more occasions would be helpful.

It should be pointed out that of the 20 known gestational diabetic women recruited in the present study, eight (40%) had no known risk factors for gestational diabetes. This figure is remarkably similar to that reported by O'Sullivan et al.[2] in a population-based study and reaffirms the necessity to screen all pregnant women for this condition.

We conclude that the 50 gm, 1-hour diabetes screening test may be administered to pregnant women who are not fasting if a threshold of 130 mg/dl, and possibly 135 or 140 mg/dl, is used. However, if a higher threshold is to be used, patients should be tested in the fasting state to avoid missing the diagnosis of gestational diabetes in a significant number of individuals.

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Decreased fetal cord prolactin concentration in diabetic pregnancies

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Infants of diabetic mothers are known to have a greater incidence of respiratory distress syndrome than normal control infants. Fetal lung maturation is modulated by a large number of hormones. To further investigate a possible role of hormonal modulators of lung maturation in infants of diabetic mothers, fetal cord prolactin, estrone, estradiol, thyroxine, triiodothyronine, and triiodothyronine-resin uptake index levels were measured in infants of diabetic mothers (n = 40) and nondiabetic mothers (n = 40) at term. Infants of diabetic mothers had significantly lower mixed-cord serum prolactin levels ($p < 0.0005$) than control infants. There was no significant difference in cord serum thyroxine, triiodothyronine-resin uptake index, triiodothyronine, estrone, or estradiol levels between the infants of diabetic mothers and the infants of control mothers. These findings raise the possibility that decreased fetal prolactin levels may be associated with, or contribute to, the delayed lung maturation reported with diabetic pregnancies. (AM J OBSTET GYNECOL 1986;154:1035-8.)

Key words: Diabetes, prolactin, pregnancy

The newborn respiratory distress syndrome contributes significantly to the increased neonatal morbidity and mortality seen in infants of diabetic mothers. Until 38.5 weeks' gestation, infants of diabetic mothers are at higher risk for respiratory distress syndrome than infants of nondiabetic mothers without perinatal disease.[1] Fetal lung maturation is modulated by a large number of hormones including insulin, cortisol, testosterone, estradiol, thyroxine, triiodothyronine, and prolactin.[2-4] The newborn infant of a diabetic mother is known to be in a hyperinsulinemic state that might delay lung maturation.[2] To further investigate possible hormonal effects on lung maturation in infants of diabetic mothers, fetal cord prolactin, estrone, estradiol, thyroxine, triiodothyronine-resin uptake index, and triiodothyronine levels were measured in infants of diabetic (n = 40) and nondiabetic mothers (n = 40) at term.

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Material and methods

Forty-two consecutive insulin-requiring diabetic mothers with singleton pregnancies delivered by D. H. S. served as the experimental population. All mothers were cared for at the Joslin Clinic, Boston, Massachusetts, with use of previously published protocols.[5] Twenty-two male and twenty female infants were delivered. The last two male infants delivered were eliminated from the experimental group in order to balance group number. A reference group of 64 cord bloods obtained from normal parturients with no perinatal disease who delivered between 36 and 39 weeks' gestation served as the control pool. Controls were chosen from this pool in order to match infant sex, route of delivery, and gestational age (within 1 week) with the experimental group.

Table I. Gestational age at birth, birth weight, amniotic fluid lecithin/sphingomyelin ratio, and saturated phosphatidylcholine level immediately before birth (mean ± SD)

	n	*Gestational age (wk)*	*Birth weight (gm)*	*Lecithin/sphingomyelin ratio*	*Saturated phosphatidylcholine (μg/dl)*
Infants of diabetic mothers					
Male	20	37.7 ± 1.25*	3527 ± 582†	3.86 ± 1.2*	1488 ± 425*
Female	20	37.4 ± 1.55*	3403 ± 568†	4.62 ± 2.3*	1395 ± 483*
Control infants					
Male	20	37.4 ± 1.55*	3419 ± 297†		
Female	20	38.1 ± 0.73*	3127 ± 332†		

*No significant difference among groups.
†Infants of diabetic mothers versus control infants, $p < 0.05$.

Table II. Maternal age, hemoglobin A_1 level in the third trimester, and White class of diabetes in diabetic and control mothers (mean ± SD)

	n	*Age (yr)*	*Hemoglobin A_1 (%)*	*Class of diabetes (n)*				
				Gestational	*B*	*C*	*D*	*F,R*
Diabetic mothers								
Male infants	20	26.2 ± 4.2	7.4 ± 1.4	1	5	5	7	2
Female infants	20	27.2 ± 4.1	7.3 ± 1.2	2	7	2	6	3
Control mothers								
Male infants	20	21.8 ± 3.7						
Female infants	20	22.4 ± 4.6						

Serum prolactin, unconjugated estrone, estradiol, thyroxine, triiodothyronine-resin uptake, triiodothyronine, amniotic fluid lecithin/sphingomyelin ratio, and saturated phosphatidylcholine were measured as previously described.[6-8] Amniotic fluid was obtained by amniocentesis from all diabetic pregnancies within the 72 hours preceding delivery. Maternal hemoglobin A_1 was measured in the last trimester of pregnancy in all diabetic pregnancies. Maternal diabetes was classified according to the system of White.[9] Statistical analysis was performed with use of one-way and two-way analysis of variance.

Results

Gestational age at birth, birth weight, lecithin/sphingomyelin ratio, saturated phosphatidylcholine level, maternal age, White classification of maternal diabetes, and maternal hemoglobin A_1 levels in the third trimester are summarized in Tables I and II. The diabetic and control pregnancies were well matched for gestational age at birth. The infants of diabetic mothers had a significantly higher birth weight than the control infants ($p < 0.05$) (Table I).

Mixed-cord serum prolactin concentration was significantly lower in infants of diabetic mothers than in control infants ($p < 0.0005$) (Fig. 1). In the infants of diabetic mothers there was no significant correlation between cord prolactin concentration and amniotic fluid lecithin/sphingomyelin ratio or saturated phosphatidylcholine level. There was no significant difference in prolactin concentration between male and female infants (Fig. 1). There was no significant difference in mixed-cord serum levels of estrone or estradiol (Table III) or in mixed-cord serum concentration of thyroxine, triiodothyronine-resin uptake index, and total triiodothyronine between the infants of diabetic mothers and the control infants (Table IV). Levels of estradiol were significantly greater in male infants (controls plus infants of diabetic mothers) versus female infants (controls plus infants of diabetic mothers) ($p < 0.05$) (Table III).

Comment

In pregnancy three separate prolactin compartments are present. Maternal circulating prolactin is controlled by the maternal pituitary. Amniotic fluid prolactin is probably regulated by the decidua and membranes, while fetal prolactin is controlled by the fetal pituitary. Luciano and Varner[10] have reported that amniotic fluid prolactin concentrations in infants of diabetic mothers are the same as in nondiabetic controls. In this study we demonstrate that fetal mixed-cord prolactin concentrations are significantly decreased at term in infants of diabetic mothers.

The decreased levels of prolactin noted in the infants of diabetic mothers may result in delayed lung matu-

Table III. Estrone and estradiol concentrations in the mixed cord serum of infants of diabetic mothers and control infants (mean ± SD)*

	n	*Estrone (ng/ml)*	*Estradiol (ng/ml)*
Infants of diabetic mothers			
Male	20	9.11 ± 0.87	6.74 ± 0.73
Female	20	8.31 ± 1.4	4.35 ± 0.49
Control infants			
Male	20	9.99 ± 1.39	6.68 ± 0.63
Female	20	9.78 ± 1.08	5.98 ± 0.69

*No significant difference in estrone or estradiol levels between infants of diabetic mothers and control infants. Estradiol concentrations were significantly elevated in male infants (infants of diabetic mothers plus controls) versus female infants (infants of diabetic mothers plus controls) ($F = 5.8_{3,76}$; $p < 0.05$).

Table IV. Thyroxine, triiodothyronine-resin uptake index, and total triiodothyronine concentrations in the mixed-cord serum of infants of diabetic mothers and control infants

	n	*Thyroxine (μg/dl)*	*Triiodothyronine-resin uptake index*	*Total triiodothyronine (ng/dl)*
Infants of diabetic mothers				
Male	20	9.31 ± .22	0.84 ± .015	56 ± 1.5
Female	20	8.73 ± .33	0.86 ± .017	48 ± 1.5
Control infants				
Male	20	9.27 ± .21	0.83 ± .019	58 ± 3.0
Female	20	9.40 ± .40	0.84 ± .015	50 ± 3.3

*No significant differences between groups.

ration. Laboratory and clinical studies suggest that fetal prolactin may play an important role in lung maturation. Fetal levels of prolactin rise markedly during the third trimester of gestation, coincident with increased production of surfactant.[11] Hamosh and Hamosh[12] have reported enhanced synthesis of phospholipids in fetal rabbits after treatment with prolactin, suggesting that prolactin may be important in regulating lung maturation.[12] Additional circumstantial evidence exists which suggests that prolactin may play a significant role in fetal lung maturation. This includes the fact that the incidence of respiratory distress syndrome has been shown to be significantly higher in premature infants with lower serum prolactin concentrations.[13] Additional support for this proposed action of prolactin is provided by the fact that the incidence of respiratory distress syndrome in premature infants born of heroin addicts is markedly reduced.[14] Opiates, for example, morphine, are potent stimuli for prolactin secretion.

Birth weight has been shown to correlate positively with prolactin concentration.[15] The mean birth weight of our infants of diabetic mothers was significantly higher than that of the controls. Therefore, based solely on these birth weight differences, we would have expected higher prolactin levels in the infants of diabetic mothers rather than the lower levels we observed. Estrogens stimulate the secretion of prolactin. No difference was observed in the levels of estradiol or estrone in infants of diabetic mothers compared to controls (Table III). Levels of estradiol were significantly greater in male infants (controls plus infants of diabetic mothers) versus female infants (controls plus infants of diabetic mothers). This finding has been previously reported by Penny et al.[16] These results suggest that differences in estradiol cannot account for the lower prolactin concentrations we observed in the infants of diabetic mothers.

There was no significant correlation between prolactin concentration and two biochemical parameters of fetal lung maturity, amniotic fluid lecithin/sphingomyelin ratio and saturated phosphatidylcholine level. Since all amniotic fluid samples were obtained at term, it is likely that the critical period (?28-34 weeks) during which hormonal influences play a major role in lung maturation had passed. Measurement of prolactin in mixed cord serum from infants of diabetic mothers of <34 weeks' gestation would be the best way to address this issue. However, improvements in perinatal care, as well as our technologic inability to obtain routinely cord blood samples in utero, makes such a study currently unlikely.

The increased incidence of respiratory distress syndrome in infants of diabetic mothers has long been a major factor in the increased morbidity and mortality observed in pregnancies complicated by diabetes. The physiologic basis of this remains unknown. The inter-

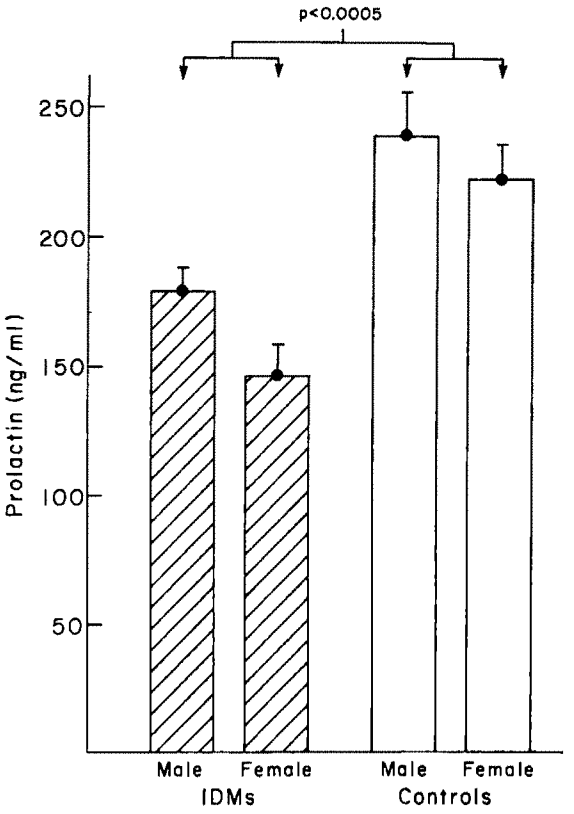


Fig. 1. Mixed-cord serum prolactin concentrations in infants of diabetic mothers *(IDMs)* and in control infants. (Mean ± SEM; n = 20 in each group.)

action of several hormones including prolactin may be a prerequisite to the normal development of the lung. Further study into the role of prolactin in lung maturation in diabetic pregnancies is warranted.

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Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers

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Hyperplasia of testicular Leydig cells and ovarian theca-lutein cells is a common histologic finding in infants of diabetic mothers. The functional correlates of this histologic finding were investigated by measurement of the β-subunit of human chorionic gonadotropin, testosterone, dihydrotestosterone, androstenedione, estradiol, and estrone in the amniotic fluid compartment of gestations with male and female fetuses in diabetic mothers (N = 34) and control women (N = 34) at term. When compared with those of control subjects, gestations of diabetic mothers had significantly higher amniotic fluid concentrations of the β-subunit of human chorionic gonadotropin. Gestations with either male or female fetuses in diabetic mothers had significantly higher amniotic fluid testosterone and dihydrotestosterone levels when compared with those of their respective gender controls. In gestations with male fetuses in diabetic mothers there was a significant positive correlation between the β-subunit of human chorionic gonadotropin and testosterone. There was no significant difference in amniotic fluid androstenedione, estradiol, or estrone levels between the gestations of diabetic mothers and those of control women. These results suggest that the testicular Leydig cell and ovarian theca-lutein cell hyperplasia seen in infants of diabetic mothers is due, in part, to elevated levels of human chorionic gonadotropin and is associated with elevated testosterone and dihydrotestosterone concentrations in the amniotic fluid. (Am J Obstet Gynecol 1986;154:1039-43.)

Key words: Diabetes, β-hCG, androgens

Leydig cell hyperplasia is a common pathologic finding in male infants of diabetic mothers.[1, 2] In female infants of diabetic mothers, an increased number of ovarian theca-lutein cells has been reported.[1, 2] The functional correlates of these abnormal histologic findings were investigated by measuring the β-subunit of human chorionic gonadotropin (β-hCG), testosterone, dihydrotestosterone, androstenedione, estradiol, and estrone in the amniotic fluid of gestations of diabetic mothers at term. Normal parturient women undergoing amniocentesis at term before repeat cesarean section served as the control population.

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Material and methods

Thirty-five consecutive insulin-requiring diabetic mothers with singleton pregnancies, who were delivered at Brigham and Women's Hospital, served as the experimental population. All mothers were cared for at the Joslin Clinic according to previously published protocols.[3] At 38 weeks' gestation the pregnant diabetic women underwent amniocentesis for measurement of the lecithin-sphingomyelin ratio (L/S ratio) and saturated phosphatidylcholine so that fetal lung maturity could be determined. For the diabetic women, gestational age was determined by the synthesis of menstrual dates, early sizing, and ultrasound examinations in the first and second trimesters. In the diabetic population 18 male and 17 female infants were delivered. The last male infant delivered was eliminated in order to balance group number.

The control population consisted of 36 women with normal pregnancies who planned to undergo repeat cesarean section. Because of unclear menstrual dates, lack of early prenatal care, and/or lack of early ultrasound dating, these women were scheduled for amniocentesis before delivery. Approximate gestational ages were estimated by synthesis of sizing at first prenatal

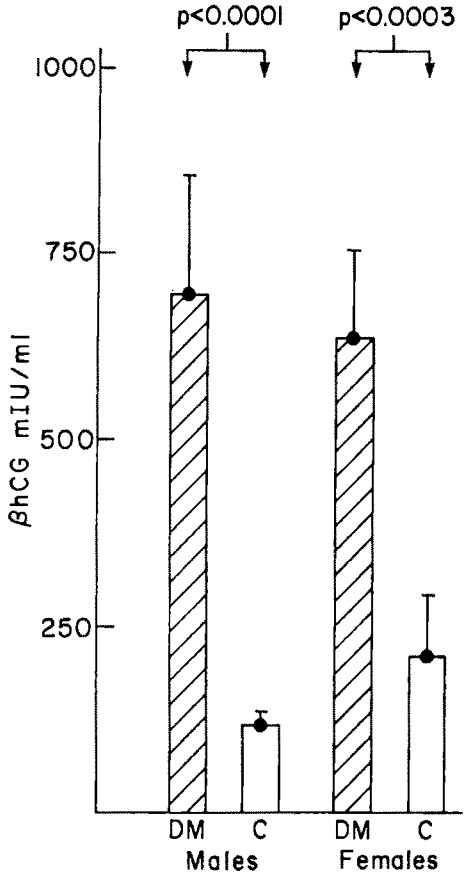


Fig. 1. Amniotic β-hCG concentrations in gestations with male and female fetuses in diabetic *(DM)* and control *(C)* mothers. N = 17 for each group. Values are mean (milli–international units per milliliter) ± SEM.

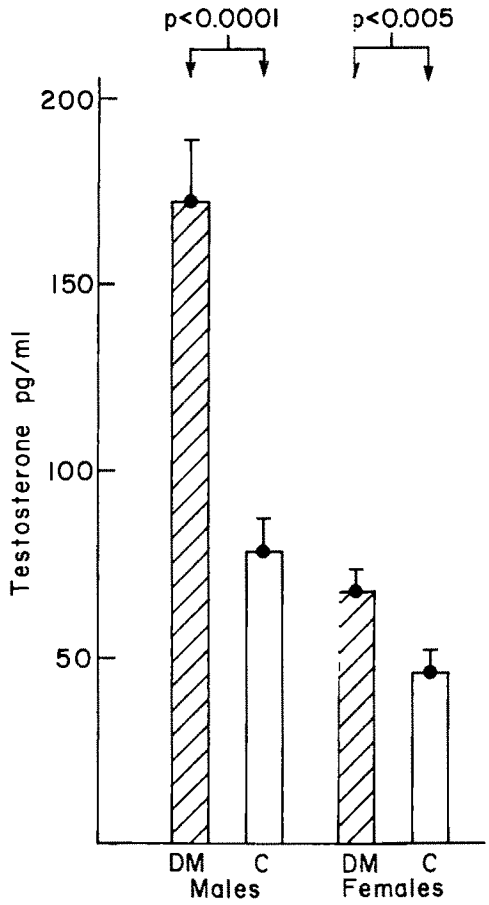


Fig. 2. Amniotic fluid testosterone concentrations in gestations with male and female fetuses in diabetic *(DM)* and control *(C)* mothers. N = 17 for each group. Values are mean (milli–international units per milliliter) ± SEM.

visit and earliest available ultrasound results. Nineteen female and 17 male infants were delivered. The last two female infants were eliminated in order to balance group number. All amniocenteses were performed under ultrasound guidance. For the experimental studies 4 ml of amniotic fluid was obtained. Sex assignments were determined at birth by an examination of the external genitalia.

Testosterone, dihydrotestosterone, androstenedione, estradiol, and estrone were measured as previously described.[4, 5] In brief, 3 ml of amniotic fluid was centrifuged at 1000 × g for 20 minutes to remove particulate and cellular debris. The supernatant was serially extracted with hexane. The steroids in the hexane extract were fractionated by Celite chromatography. Steroids were measured by radioimmunoassay with the use of specific antibodies. Tritiated steroids purified by Celite chromatography were added to the samples before extraction in order to assess recovery. Recoveries ranged from 70% to 95%. Results were scaled for recovery losses.

Amniotic fluid β-hCG was measured with the use of a double-antibody method as previously described.[6] A highly specific anti–β-hCG antibody was obtained from Serono (Braintree, Massachusetts). Human chorionic gonadotropin (hCG) (second international reference preparation) was used as the reference preparation. hCG (Cr-121) was used for iodination. The hCG was provided by the National Hormone and Pituitary Program.

The amniotic fluid L/S ratio and saturated phosphatidylcholine were measured as previously described. Maternal hemoglobin A_1 was measured in the last trimester of pregnancy in all the diabetic pregnancies. Maternal diabetes was classified according to the system of White. Statistical analysis was performed by the use of one-way analysis of variance.

Results

Gestational age at amniocentesis was significantly lower ($p < 0.05$) in the gestations of diabetic mothers (male fetuses, 38.1 ± 0.77 weeks; female fetuses, 38.0 ± 0.56 weeks; mean ± SD, N = 17 for each group) than in the control gestations (male fetuses,

Table I. Dihydrotestosterone, androstenedione, estradiol, and estrone concentrations in amniotic fluid of gestations of diabetic mothers and control mothers

Gestations	*Dihydrotestosterone (pg/ml)*	*Androstenedione (pg/ml)*	*Estradiol (pg/ml)*	*Estrone (pg/ml)*
Male fetuses				
Diabetic mothers	29.4 ± 1.97*	670 ± 53.8†	145 ± 21.4†	611 ± 51.9†
Control mothers	21.8 ± 1.85*	620 ± 82.0†	115 ± 13.7†	561 ± 60.5†
Female fetuses				
Diabetic mothers	21.6 ± 2.67‡	571 ± 78.0†	126 ± 19.1†	615 ± 83.8†
Control mothers	16.2 ± 1.57‡	527 ± 71.9†	111 ± 10.7†	602 ± 57.4†

Values are mean ± SEM. N = 17 for each group.
*$p < 0.006$.
†No significant difference between groups.
‡$p < 0.05$.

38.9 ± 0.81 weeks; female fetuses, 38.6 ± 0.77 weeks). The range of gestational age at amniocentesis for the diabetic and control pregnancies was 36.7 to 40 and 38 to 40 weeks, respectively. Since the control pregnancies were composed of a group of women with uncertain menstrual dates and/or a late first prenatal visit the accuracy of the gestational age determination is difficult to assess. There was no significant correlation ($p > 0.05$) between gestational age at amniocentesis and β-hCG, testosterone, dihydrotestosterone, androstenedione, estradiol, or estrone levels in any group.

Birth weight was not significantly different ($p > 0.05$) between the infants of diabetic mothers (male, 3675 ± 559 gm; female, 3343 ± 510 gm; mean ± SD, N = 17 for each group) and the infants of control mothers (male, 3564 ± 332 gm; female, 3202 ± 372 gm). There was no statistically significant difference in the amniotic fluid L/S ratio between the diabetic gestations (male, 3.9 ± 1.17; female, 4.2 ± 1.90; mean ± SD, N = 17 for each group) and the control gestations (male, 4.7 ± 1.53; female, 4.1 ± 1.71). Similarly, there was no statistically significant difference in the amniotic fluid saturated phosphatidylcholine concentrations between the diabetic gestations (male, 1544 ± 434 μg/dl; female, 1487 ± 448 μg/dl; mean ± SD, N = 17) and the control gestations (male, 1490 ± 441 μg/dl; female, 1180 ± 538 μg/dl).

In the third trimester of pregnancy, maternal serum hemoglobin A_1 was 7.5% ± 1.4% (mean ± SD) in the diabetic mothers with male fetuses and 7.1% ± 1.1% (mean ± SD) in the diabetic mothers with female fetuses. The distribution of the diabetic mothers in the White classification was: gestational, four; Class B, 12; Class C, four; Class D, 11; and Class F to R, three.

The amniotic fluid β-hCG level was significantly higher in the diabetic gestations than in the control gestations ($p < 0.0001$) (Fig. 1). Analysis of relevant subgroups revealed that gestations with male fetuses in diabetic mothers had significantly higher amniotic fluid β-hCG levels than gestations with male fetuses in control mothers ($p < 0.0001$). Gestations with female fetuses in diabetic mothers had significantly higher amniotic fluid β-hCG levels than gestations with female fetuses in control mothers ($p < 0.0003$).

Gestations with male fetuses in diabetic mothers had significantly higher amniotic fluid testosterone levels than gestations with male fetuses in control mothers ($p < 0.001$) (Fig. 2). Gestations with female fetuses in diabetic mothers had significantly higher amniotic fluid testosterone levels than gestations with female fetuses in control mothers ($p < 0.005$). Amniotic fluid testosterone levels were similar in gestations with female fetuses in diabetic mothers and gestations with male fetuses in control mothers ($F = 0.60$, NS).

Amniotic fluid dihydrotestosterone concentrations were significantly higher in gestations with male fetuses ($p < 0.006$) and female fetuses ($p < 0.05$) in diabetic mothers when compared with levels in their respective gender controls (Table I). Amniotic fluid dihydrotestosterone concentrations were similar in gestations with female fetuses in diabetic mothers and gestations with male fetuses in control mothers ($F = 0.004$, NS).

In the gestations with male fetuses in diabetic mothers there was a statistically significant positive correlation between the amniotic fluid concentration of β-hCG and both testosterone ($r = 0.473$, $p < 0.05$) and dihydrotestosterone ($r = 0.585$, $p < 0.01$). In gestations with female fetuses in diabetic mothers and in control gestations (both male and female fetuses) there was no significant correlation between levels of β-hCG and testosterone or dihydrotestosterone. In the gestations with male and female fetuses in diabetic mothers there was no significant correlation between amniotic fluid concentrations of β-hCG, testosterone, or dihydrotestosterone and maternal insulin requirements, class of diabetes, or hemoglobin A_1 in the third trimester. In the gestations with male fetuses in diabetic mothers there was a significant correlation between birth weight and β-hCG ($r = 0.51$, $p < 0.05$), but in the gestations with female fetuses in diabetic mothers there was no sig-

nificant correlation between birth weight and β-hCG level.

Gestations of diabetic mothers and control gestations had similar amniotic fluid concentrations of androstenedione, estradiol, and estrone (Table I).

Comment

The results presented here demonstrate that β-hCG, testosterone, and dihydrotestosterone concentrations are significantly elevated in the amniotic fluid of gestations with both male and female fetuses in diabetic mothers at term as compared with those of appropriate gender controls. These differences must be interpreted with caution since the control and diabetic populations may differ by factors other than the diabetic state. For example, the control patients required amniocentesis before repeat cesarean delivery because of uncertain menstrual dates and/or late first prenatal visit. These control patients may differ significantly from the diabetic patients with respect to socioeconomic class, nutritional factors, or other characteristics. The impact of these possible differences was not evaluated in the current study.

The sources of amniotic fluid testosterone are poorly characterized, but in gestations with male fetuses a major source of amniotic fluid testosterone is probably urinary excretion of testosterone derived from the fetal testes.[7] The observation that gestations with male fetuses in diabetic mothers have elevated amniotic fluid testosterone concentrations correlates well with the pathologic observation that male infants of diabetic mothers have Leydig cell hyperplasia.[1] Hultquist and Olding[1] reported that quantitative histologic examination of the testes of male infants of diabetic mothers revealed a fourfold increase in Leydig cells per unit volume of testicular tissue when compared with the testes of infants of nondiabetic mothers.[1] In the Hultquist and Olding study[1] the total testicular weight was equivalent in the infants of diabetic and nondiabetic mothers. This finding supports the hypothesis that in male infants of diabetic mothers Leydig cell hyperplasia does not represent "nonspecific" organomegaly.

The stimuli that induce Leydig cell hyperplasia and increased amniotic fluid testosterone in gestations with male fetuses in diabetic mothers are not clear. However, both hCG[8] and insulin[9] may participate in this process. The amniotic fluid β-hCG concentrations measured in this study reflect both intact hCG and free β-hCG. At term, intact hCG is present in amniotic fluid at concentrations that are significantly higher than those of free β-hCG. Therefore, the β-hCG concentrations measured in this study probably reflect, in large measure, intact circulating hCG. The significant correlation between β-hCG and amniotic fluid testosterone supports the hypothesis that the Leydig cell hyperplasia is caused by elevated biologically active hCG. Insulin may also play a role in the pathogenesis of the Leydig cell hyperplasia of male infants of diabetic mothers. Gestations of diabetic mothers are characterized by hyperinsulinemia in the fetal compartment, and recent evidence suggests that insulin may directly stimulate Leydig cell growth and steroidogenesis.[9]

The normal human fetal ovary is not as steroidogenically active as the fetal testis.[7] However, in female infants of diabetic mothers ovarian theca-lutein hyperplasia has been observed.[1] This pathologic observation correlates well with the functional observation that amniotic fluid testosterone and dihydrotestosterone levels are elevated in female gestations of diabetic mothers. Both hCG and insulin[10] may participate in the ovarian theca-lutein hyperplasia. The presence of ovarian theca-lutein hyperplasia in female infants of diabetic mothers may have implications for the adult ovarian functions. It is conceivable that when these infants become adults the perinatal ovarian theca-lutein hyperplasia might result in a greater incidence of anovulation and ovarian hyperandrogenism as compared with that in control women.

The mechanisms that result in increased amniotic fluid β-hCG levels in gestations of diabetic mothers at term are unknown. Possible mechanisms include abnormalities in hCG production or metabolism or a disruption in the processes that maintain the high maternal to amniotic fluid hCG gradient (100 to 1). Diabetic pregnancies are known to be associated with hyperplacentosis.[2] Placental weight was not measured in this study, and therefore it is not possible to evaluate hyperplacentosis as a cause of elevated amniotic fluid β-hCG levels. However, birth weight correlates with placental weight, and in this study there was a significant correlation between birth weight and amniotic fluid β-hCG level in the male fetus ($r = 0.51$, $p < 0.05$) but not in the female fetus ($r = 0.08$ NS) in gestations of diabetic mothers. This would suggest that at least in gestations with female fetuses in diabetic mothers the elevated amniotic fluid β-hCG level is not due to macrosomia.

Other investigators have reported that amniotic fluid and maternal hCG levels are significantly elevated at term in pregnancies complicated by pregnancy-induced hypertension.[11] In our study, diabetic pregnancies complicated by pregnancy-induced hypertension (diagnosed by lateral recumbent diastolic blood pressure >90 mm Hg with no prepregnancy or postpartum evidence of hypertension) had higher amniotic fluid β-hCG concentrations (982 ± 187 mIU/ml, mean ± SEM, $n = 11$) than diabetic pregnancies not complicated by pregnancy-induced hypertension (516 ± 97.4 mIU/ml, $n = 23$, $p < 0.03$). However, the diabetic pregnancies not complicated by pregnancy-in-

duced hypertension also had significantly greater amniotic fluid β-hCG levels than those of normal control pregnancies (516 ± 97.4 versus 166 ± 41.7 mIU/ml, $p < 0.0001$). Therefore, pregnancy-induced hypertension and maternal diabetes are probably independent but interacting causes of elevated β-hCG at term.

The physiologic implications of elevated amniotic fluid testosterone and dihydrotestosterone concentrations in gestations of diabetic mothers are not clear. However, normal fetal lung maturation is dependent on the fetal concentration of a large number of hormones: insulin, cortisol, testosterone, prolactin, thyroxine, and estradiol.[12] At any given gestational age significantly more male infants have respiratory distress syndrome than female infants.[13] There is substantial evidence that this sex difference in lung maturity is due in part to differences in circulating androgens.[14] Elevated androgens in male and female fetuses of diabetic mothers may slow the rate of lung maturation in these fetuses and contribute to the observed increase in respiratory distress syndrome in infants of diabetic mothers.[15, 16]

In summary, elevated levels of hCG in diabetic pregnancies may contribute to testicular Leydig cell and ovarian theca-lutein cell hyperplasia. These structural abnormalities are paralleled by the functional observation that amniotic fluid testosterone and dihydrotestosterone concentrations are elevated in gestations of diabetic mothers.

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Hypertensive diseases of pregnancy and parity

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Chesley's classic long-term follow up study of eclamptic women clearly demonstrated the prognostic significance of parity in the differential diagnosis of various hypertensive diseases of pregnancy. Multiparous patients with eclampsia were different on long-term follow-up from primiparous eclamptic women. A logical conclusion from this observation is that multiparous and primiparous patient groups should be analyzed separately whenever hypertensive diseases of pregnancy are evaluated. This study is therefore an attempt to define the clinical profile of hypertension during pregnancy on the basis of parity alone. The prenatal and hospital records of 99 successive pregnant patients identified as hypertensive during a 1-year period at Mount Sinai Hospital Medical Center served as the study population. A group of 25 primiparous and 25 multiparous patients, all with uncomplicated pregnancies and normal deliveries in sequence during a 1-month period, served as normal nonhypertensive control groups. Various pregnancy parameters were statistically evaluated for study and control groups. Significant differences were found between hypertensive primiparous and multiparous patients in mean weight increase ($p < 0.05$), gestational age at first increase in blood pressure ($p < 0.007$), and time from first increase in blood pressure until delivery ($p < 0.008$). The difference in birth weight was not significant between hypertensive groups. In contrast, among the control groups, multiparous patients had significantly larger offspring than primiparous patients ($p < 0.01$). Weights of normal primiparous control women were almost identical to those of primiparous hypertensive women (3252.8 ± 511.8 versus 3203.26 ± 679.5 gm). Birth weights of offspring from multiparous hypertensive women, while not significantly different from those of primiparous hypertensive women, were significantly lower than those of multiparous control subjects (3093.94 ± 898.7 versus 3593 ± 305.6; $p < 0.01$). No significant differences in a variety of laboratory findings, reflexes, edema, or mode of delivery were observed between the various groups. The clinical and laboratory presentation of hypertension in pregnancy may be similar between primiparous and multiparous patients. Nevertheless, clear differences do exist in both maternal presentation and impact of maternal disease on fetal growth and development. These differences strongly suggest a different pathophysiology as the underlying cause of hypertensive disease in primiparous and multiparous pregnant women. Future studies of hypertensive diseases of pregnancy therefore need to be designed accordingly. (AM J OBSTET GYNECOL 1986;154:1044-9.)

Key words: Pregnancy hypertension, eclampsia, parity

Hypertensive diseases represent one of the most common among medical complications of pregnancy with a reported incidence of 6% to 30%.[1, 2] They also represent a major cause of maternal and fetal as well as neonatal morbidity and mortality.[2, 3] The literature abounds with studies devoted to the investigation of many different factors concerning cause, diagnosis, and management of hypertensive diseases in pregnancy.[4, 5] However, terminology as well as diagnostic criteria for various hypertensive diseases has remained vague and somewhat arbitrary in all systems of classification devised so far. It is particularly worrisome that most studies do not specifically refer to disease parameters in correlation with parity of investigated patient populations. This is despite Chesley's classic long-term follow-up study of eclamptic women, which clearly demonstrated the prognostic significance of parity in the differential diagnosis of various hypertensive diseases of pregnancy.[6] By prospective evaluation up to 1974 of 220 women who survived eclampsia between 1931 and 1951, Chesley was able to demonstrate the importance of parity in the differentiation between eclamptic patients and those he designated as having superimposed eclampsia. He chose to investigate eclamptic rather than preeclamptic patients because the diagnosis of eclampsia seemed more reliable than the diagnosis of the milder preeclampsia. Nevertheless, he recognized that even the diagnosis of eclampsia at times was made in error.[6] While the study was in progress, Chesley

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Table I. Study populations

	Hypertensive group					*Control group*				
	Primiparous (N = 46)		*Multiparous (N = 53)*			*Primiparous (N = 25)*		*Multiparous (N = 25)*		
	Mean	*SD*	*Mean*	*SD*	*p**	*Mean*	*SD*	*Mean*	*SD*	*p**
Age (yr)	18.9	4.0	27.3	5.5	0.0005	20	4.4	25.5	5.1	0.001
Weight increase (pounds)	24.4	10.0	19.9	9.6	0.05	—	—	—	—	—
First increase in MAP† (mm Hg)	102.6	10.3	103.0	12.9	NS	—	—	—	—	—
Gestational age at first MAP increase (wk)	35.9	4.9	31.4	7.7	0.007	—	—	—	—	—
MAP on admission (mm Hg)	113.2	16.5	111.4	14.0	NS	—	—	—	—	—
Gestational age at delivery (wk)	37.9‡	2.5	37.5§	3.4	NS	39.5‡	1.4	40.2§	0.4	NS
Time, first MAP increase to delivery‖ (wk)	2.1	4.5	6.5	8.6	0.008	—	—	—	—	—
Birth weight (gm)	3203.3‡	679.5	3093.9‡	898.7	NS	3252.8‡	511.8	3593.0‡	305.6	0.01
White blood cell count (thousand/mm³)	10.3	3.1	10.2	3.0	NS	12.0	2.6	10.1	3.0	NS
Hemoglobin (gm/dl)	11.6	1.6	12.3	1.6	NS	11.4	1.4	11.9	1.0	NS
Hematocrit (%)	34.3	4.4	36.3	4.2	0.024	33.8	4.0	35.6	3.2	NS
Platelets (thousand/mm³)	234.7¶	64.0	229.8§	83.0	NS	264.8¶	60.0	281.0§	55.8	NS
Uric acid (mg/dl)	5.6	5.5	5.6	5.5	NS	—	—	—	—	—

For detailed statistical comparison between study and control groups see text. Significant differences are shown. NS = Not significant.

*Student's *t* test.

†MAP = Mean arterial pressure.

‡$p < 0.01$.

§$p < 0.001$.

‖Difference in gestational age from first increase in blood pressure until delivery.

¶$p < 0.02$.

noted that the majority of patients (75%) were primigravid and started a separate analysis of those patients from the remaining multigravid women. This then allowed him to demonstrate that the primigravid group of eclamptic patients and the control subjects had an equal chance to develop chronic hypertensive disorders on follow-up. In contradistinction, the multiparous group clearly demonstrated on follow-up an increased prevalence of underlying disease, which, in fact, led to a significant increase in remote deaths in this particular group as compared with both control subjects and primigravid eclamptic women.[6] Chesley himself rescinded a number of earlier publications based on these findings as he concluded that "any study of preeclampsia that includes multiparous patients will lead to erroneous conclusions."[6] Despite Chesley's general recognition as a pioneer in the investigation of hypertensive diseases in pregnancy, this recommendation has been largely ignored by the medical community. The issue is further complicated by the fact that since his recommendations were made some investigators have suggested that preeclampsia may occur in multigravid women if a new paternity has been established.[7] Such a contention would agree with the presently popular immunologic theory of preeclampsia[8] and may represent a factual finding; however, as the documentation lacks the sophistication of Chesley's study it should at this time not be taken as a reason to contradict his basic conclusions and recommendations.

Building on Chesley's observations we previously theorized that the underlying pathophysiology of pregnancy-related hypertension in primiparous patients may vary from that of multiparous patients.[8] In order to either support or refute such a concept the study presented here was designed to define hypertensive diseases of pregnancy solely on the basis of parity. Our observation of statistically significant differences between primiparous and multiparous hypertensive pregnancies in both mother and offspring supports the contention that differences in underlying mechanisms for the development of hypertension in both pregnant disease groups exist.

Material and methods

The prenatal and hospital records of 99 successive pregnant patients identified as hypertensive during a 1-year period at Mount Sinai Hospital Medical Center served as the study population.

Hypertension was defined as blood pressure ≥120/80 mm Hg during the midtrimester and 130/90 mm Hg in the last trimester of pregnancy. The Collaborative Perinatal Project[1] influenced the choosing of these

Table II. Type of delivery in primiparous and multiparous hypertensive patients

	Type of delivery			
	Primiparous		*Multiparous*	
	n	%	*n*	%
Normal spontaneous vaginal delivery	32	69.6	40	75.5
Cesarean section	14	30.4	13	24.5
Cephalopelvic disproportion	12	85.7	9	69.2
Fetal distress	2	14.2	4	30.8

$p = 0.66$ (χ^2 after Yates' correction).

values as cutoffs as it revealed that perinatal mortality varies with maternal blood pressure and begins to rise when the maternal diastolic pressure exceeds either 75 mm Hg in the second trimester or 85 mm Hg in the third trimester.

The hypertensive study population represented 7.2% of the total obstetric population delivered during that time period. A group of 25 primiparous and 25 multiparous patients, with uncomplicated pregnancies and normal deliveries in sequence during a 1-month period, served as normal nonhypertensive control groups.

Mount Sinai Hospital Medical Center is a tertiary care institution that serves a largely poor inner city population with above average high-risk characteristics. While approximately half of all obstetric patients are private and under the care of their own obstetricians, perinatal consultation is usually obtained in high-risk situations. The obstetric management of the study was therefore rather homogeneous.

All patient data were extrapolated by one of the authors (L. R. B.) while statistical evaluation was performed by a medical statistician (M. N.). Data were evaluated by appropriate methods, which included Student's *T* test, χ^2 after Yates' correction, and Mann-Whitney U–Wilcoxon rank sum W test.

Results

Hypertensive patients. Table I summarizes differences between primiparous and multiparous hypertensive patients.

Ninety-nine patients were identified as hypertensive; 53 were multiparous with a mean age of 27, and 46 were primiparous with a mean age of 18. The age difference was statistically significant ($p < 0.0005$) The mean weight increase during pregnancy in the multiparous hypertensive group was significantly lower than in the primiparous hypertensive group, 19.9 versus 24.4 pounds ($p < 0.05$).

The mean arterial pressure at the time of first blood pressure increase was almost equal for both groups, 103 for the multiparous group versus 102.6 for the primiparous group.

The mean gestational age at first increase in blood pressure was significantly different between the two groups: 31.4 weeks for multiparous hypertensive patients and 35.9 weeks for the primiparous group ($p < 0.007$).

The mean arterial pressure on admission for labor was not statistically different with the multiparous group having a value of 111.4 versus 113.2 for the primiparous patients.

The time from the first increase of mean blood pressure to delivery was 6.5 weeks for the multiparous group versus 2.1 weeks for the primiparous patients. This difference is statistically significant ($p < 0.008$).

A total of 75.5% of the multiparous hypertensive patients were delivered vaginally and 24.5% had cesarean section. Of these cesarean sections, four were secondary to fetal distress and the remainder were due to dystocia. Of the primiparous group 69.6% were delivered vaginally and 30.4% had cesarean section delivery. Two of these were secondary to fetal distress, and the remainder were for dystocia. The difference in delivery pattern between the two groups was not statistically significant ($p = 0.66$) (Table II).

The mean gestational age at the time of delivery was 37.5 weeks for the multiparous hypertensive patients and 37.9 weeks for the primiparous patients. Although the means were not significantly different between the groups a trend toward earlier birth of infants in the hypertensive multiparous group can be seen in Fig. 1.

The mean infant birth weights were not statistically different: 3093 gm in the hypertensive group versus 3203.3 gm for the primiparous patients. Multiparous patients, however, frequently had smaller infants (see Fig. 2). This observation is even further strengthened by the fact that in the multiparous group all infants >4000 gm were products of a pregnancy with gestational diabetes. There were no cases of gestational diabetes in the primiparous group. The relationship to birth weights of offspring of normal pregnancies is discussed below.

No significant abnormalities or differences in means for white blood cell count, hemoglobin/hematocrit, or platelets were observed in hypertensive patients. Uric acid levels were mildly increased in both hypertensive groups but were not statistically different from each other.

Reflexes and edema were reported equally in both hypertensive groups.

Normal control patients. Table I also summarizes data from 25 normal primiparous and 25 normal multiparous control patients. As may be seen, except for age and birth weight, no significant differences were

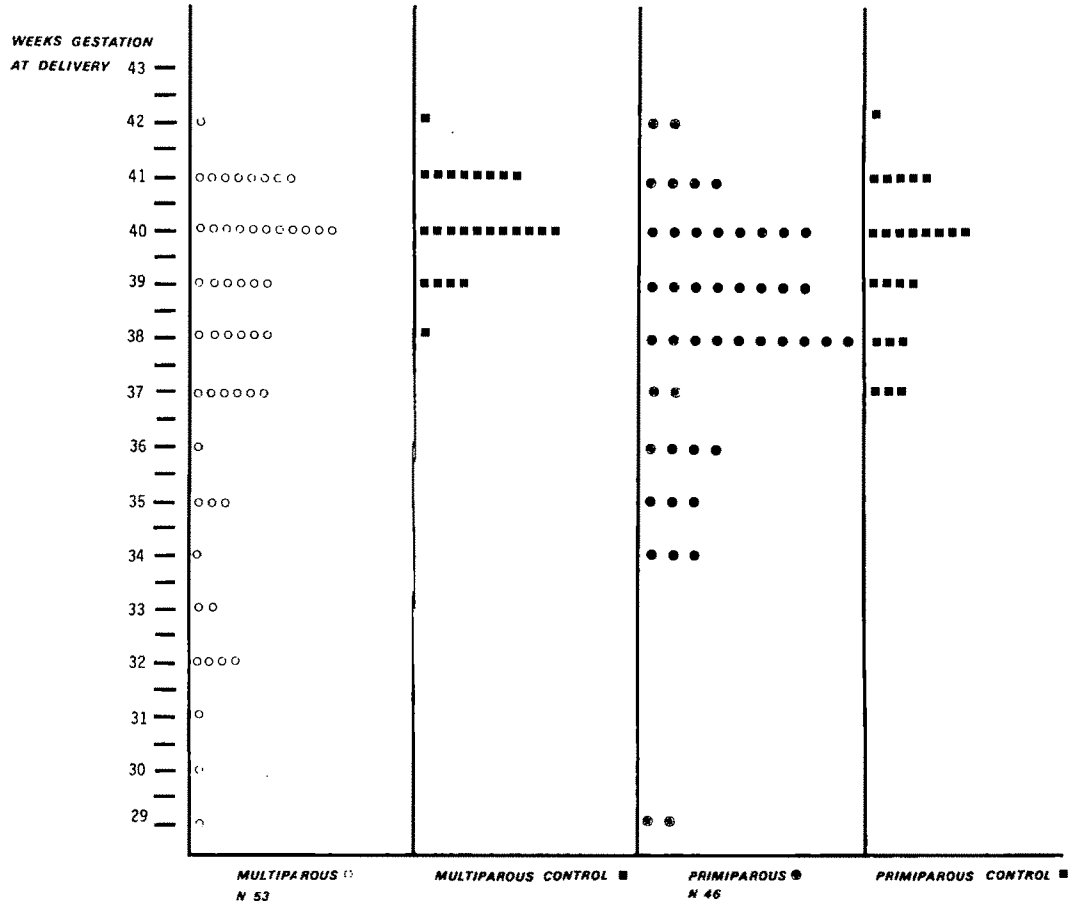


Fig. 1. Time of delivery for primiparous and multiparous hypertensive women and control patients. No statistically significant difference between groups was noted ($p = 0.50$, χ^2 after Yates' correction). A trend toward earlier delivery time for the multiparous hypertensive patients is seen.

noted between primiparous and multiparous healthy control women for gestational age at delivery, white blood cell count, hemoglobin/hematocrit, and platelet count.

Gestational age at delivery was lower in both primiparous and multiparous hypertensive patients than in normal control subjects. Primiparous hypertensive women were delivered of their infants statistically earlier than primiparous normal women at a mean gestational age of 37.9 ± 2.8 versus 39.5 ± 1.4 weeks, respectively ($p < 0.01$). Multiparous hypertensive women, who were not statistically different from primiparous hypertensive women, clearly were delivered of their infants earlier than their multiparous control counterparts (37.5 ± 3.4 versus 40.2 ± 0.4 weeks; $p < 0.001$).

Birth weights of offspring of normal multiparous women were, as expected, significantly higher than those of offspring of normal primiparous women (3593 ± 305.6 versus 3252.8 ± 511.8 gm; $p < 0.01$). Interestingly, birth weights of normal primiparous control subjects were almost identical to those of hypertensive primiparous women (3252.8 ± 511.8 versus 3203.3 ± 679.5 gm). Birth weights of offspring of hypertensive multiparous women, while not statistically different from those of offspring of hypertensive primiparous women, were however statistically clearly lower than those of offspring of multiparous control women (3093.9 ± 898.7 versus 3593 ± 305.6 gm; $p < 0.01$).

White blood cell counts and hemoglobin/hematocrit were not different in hypertensive and control patients. Platelets of both primiparous and multiparous hypertensive patients were, however, clearly lower than in their respective control subjects ($p < 0.02$ for primiparous groups; $p < 0.001$ for multiparous groups; Table I).

Comment

Mariceau's observation in 1694,[9] indicating that preeclampsia-eclampsia appeared primarily in primigravid women, is an observation that today we consider confirmed. It is indicative of our lack of progress in the

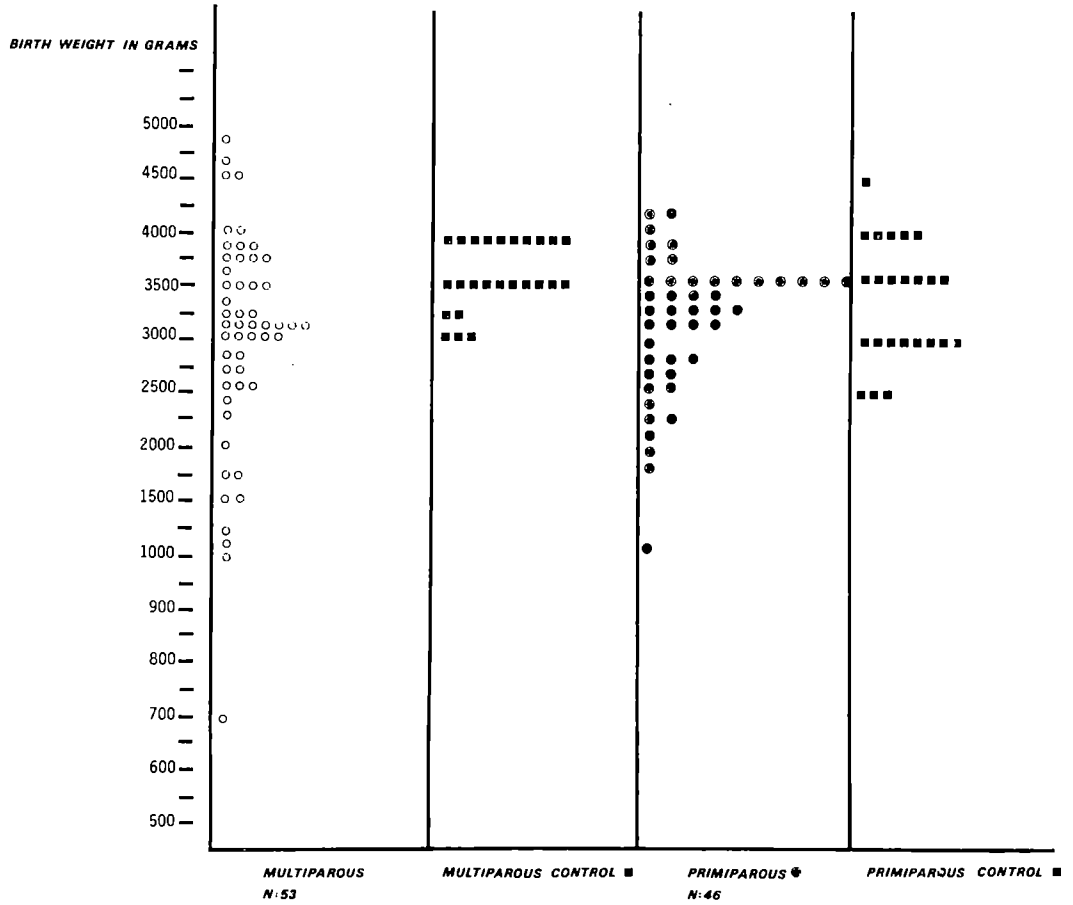


Fig. 2. Birth weights of infants from primiparous and multiparous hypertensive patients and control patients. While no statistical differences in means between groups was noted ($p = 0.45$, χ^2 after Yates' correction), multiparous control women showed the expected higher weight in comparison to that of primiparous control women while hypertensive multiparous pregnancies resulted in neonatal weights almost identical to those of multiparous pregnancies. Furthermore, all infants with weights >4000 gm born to multiparous mothers with hypertension came from pregnancies complicated by gestational diabetes while no patients in the primiparous hypertensive group had diabetes.

understanding of hypertension in pregnancy that, almost 300 years after Mariceau's observation, gravidity still represents the single best option in the differential diagnosis of hypertensive diseases during pregnancy.

The widely used term "superimposed" preeclampsia for a largely multiparous group of patients, in contrast to the overwhelmingly primiparous group of patients with "true" preeclampsia, suggests that the term preeclampsia is indicative of the same underlying pathophysiologic characteristics in either patient populations. If such a concept were to be correct then maternal clinical presentation and impact of maternal disease on pregnancy should be the same or at least very similar. The clinical difficulty in differentiating the various forms of hypertensive diseases in pregnancy has been the basis of much confusion. Considerable data, suggesting significant differences in both maternal and fetal presentation between primiparous and multiparous hypertensive pregnancies, have been recognized since Chesley's earliest studies. Chesley[10] himself noted that "superimposed" preeclampsia exhibited the most dangerous prognosis. Similarly, it has been known for at least a decade that offspring of "superimposed" hypertensive patients exhibit a considerably higher incidence of intrauterine growth retardation.[11] Clear differences in clinical presentation between primiparous and multiparous hypertensive pregnancies have thus been apparent for a considerable time. These differences, however, have been directly attributed to underlying medical conditions in the multiparous hypertensive group rather than to the underlying pathophysiologic characteristics that create hypertension in pregnancy.

By comparing primiparity to multiparity in a crossover analysis between hypertensive and normal pregnancies this study represents an attempt to control for the multiparity factor of underlying disease. If multiparity and automatically associated conditions such as

advanced maternal age are in fact responsible for the outcome differences between primiparous and multiparous hypertensive pregnancies then the crossover analysis for multiparous healthy control women should reveal some of these effects.

The study presented here, contrary to that supposition, very strongly supports the contention that different pathophysiologic mechanisms are operative in primiparous and multiparous hypertensive patients during pregnancy. While the clinical presentation at the time of the labor may in fact be very similar, as indicated by identical blood pressure elevations in labor, similar timing of delivery, and largely similar laboratory data (Table I), considerable differences in both maternal and fetal presentation were nevertheless noted. Multiparous patients exhibited a significantly earlier mean arterial pressure increase compared with that of primiparous patients ($p < 0.007$). Because the time of delivery for both hypertensive groups was almost identical, the time period between first mean arterial pressure rise and delivery was significantly longer in the multiparous group ($p < 0.008$). Maternal weight gain in the multiparous group was significantly lower ($p < 0.05$). Unfortunately, weight information was very incomplete in the two control groups and reliable weight data therefore could not be obtained for these patients.

The most convincing results from this study are those referring to fetal outcome. While the control populations are reflective of the known fact that birth weights increase with parity[12] this relationship was not observed between the two hypertensive groups. In fact, the multiparous hypertensive group had a lower (although not significant) mean birth weight than the primiparous group of hypertensive mothers. Moreover, while primiparous hypertensive women had only a 49 gm lower mean birth weight than primiparous normal control women, multiparous hypertensive women had newborn infants who were 500 gm lighter in weight than offspring of multiparous normal control mothers.

This finding is most remarkable since it suggests that the nutritional support to the fetus during multiparous hypertensive pregnancy is considerably diminished as compared with that during the primiparous gestation. Nutritional support to the fetus is largely dependent on vascular perfusion of the placenta and transmission of nutrients.[13] Growth-retarded infants are usually associated with growth-retarded, calcified, and insufficient placentas. Such placental pathologic conditions have been repeatedly reported in conjunction with superimposed multiparous hypertensive diseases.[10] In contrast, classical primiparous preeclampsia has been shown to be at least initially associated with larger than normal placentas.[14] The term hyperplacentosis has in fact been coined for conditions that are associated with larger than average placental volume, all of which are associated with a higher than normal incidence of preeclampsia/eclampsia.[15] By the time delivery occurs the initially abnormally large placentas have decreased in size, often to sizes even smaller than average.[15] Nevertheless, the classically growth-retarded placentas with calcifications and infarcts are not a usual feature of primiparous preeclampsia but are fairly typical of the multiparous form of hypertensive disease in pregnancy.

The causes and/or pathophysiologic features of preeclampsia/eclampsia are still only poorly understood. The study presented here suggests that, whatever the underlying mechanisms of maternal vascular disease may be, the resultant maternal and fetal effects vary between multiparous and primiparous hypertensive pregnancies. This observation needs to be considered in reference to terminology of hypertensive diseases in pregnancy as well as in the study design of future investigations of hypertension in pregnancy.

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Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic

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The plasma fibronectin concentration was abnormally elevated (>400 μg/ml) in 16 of 17 normotensive gravid women who subsequently developed preeclampsia. Of this group, 13 had elevated levels detectable ≥4 weeks before the onset of hypertension. Our results indicate that plasma fibronectin levels can be abnormally increased long before the onset of clinical symptoms and that abnormalities of this glycoprotein may be an early indication of this pathologic process. (Am J Obstet Gynecol 1986;154:1050-2.)

Key words: Plasma fibronectin, preeclampsia, predictive value

The early suspicion of preeclampsia includes the clinical findings of abnormal edema and/or suspicious weight gain and therefore may require subjective interpretation. We have demonstrated in a previous study that plasma fibronectin concentrations are abnormally elevated in patients with preeclampsia and suggested that measurement of this glycoprotein may offer a biochemical marker for this disorder.[1] The present study was undertaken to determine the utility of plasma fibronectin levels as an indicator of normotensive gravid patients destined to become clinically preeclamptic later in pregnancy.

Material and methods

All obstetric outpatients seen at the Medical University of South Carolina routinely had blood drawn for determination of plasma fibronectin levels as part of their clinic visits. The plasma was frozen at −70° C and later assayed for plasma fibronectin as indicated. All assays were performed in a blinded fashion with the clinical diagnoses not being available to the physician performing the assay. Seventeen of these patients subsequently developed preeclampsia during the course of pregnancy. Twenty-three gravid patients with uncomplicated pregnancies were randomly selected as controls. The number of plasma samples available from any one patient varied and depended on the gestational age at which the patient was first seen in the outpatient clinic. Abnormal weight gain was defined as ≥5 pounds per week. Suspicious weight gain was defined as ≥2 pounds per week or ≥6 pounds per month. Edema was considered abnormal if described as such by the physician caring for the patient. Preeclampsia was defined by widely used criteria.[2] No patients with insulin-dependent diabetes mellitus or chronic hypertension were included in the study.

Blood samples of 10 ml were collected atraumatically by venipuncture with 21-gauge needles and transferred to test tubes containing sodium citrate anticoagulant. Platelet-poor plasma was prepared by centrifugation at 2100 × g for 15 minutes. Plasma fibronectin was measured as previously described.[1] Unless assayed that day, all samples were frozen at −70° C. This freeze-thaw step had no effect on the measurement of fibronectin concentration. Serial aliquots of an internal control plasma sample, which were thawed and assayed at weekly intervals, had a mean fibronectin concentration of 403 μg/ml ± 37 with a range of 375 to 420 μg/ml and a coefficient of variation of 4.5%. Results were analyzed with Student's *t* test.

Results

The results of the preeclamptic group in this study are shown in Table I. Seventeen patients had a diagnosis of preeclampsia made during the course of pregnancy. The mean (±SD) fibronectin concentration for this group was 597 ± 173 μg/ml. In 16 (94%) of these patients the fibronectin concentration was abnormally increased (>400 μg/ml) before the appearance of hypertension. Thirteen of these patients had elevated fibronectin levels detectable ≥4 weeks before the appearance of hypertension. In 12, the elevated fibronectin levels preceded abnormal or suspicious weight gain. Earlier fibronectin elevations cannot be ruled out in those patients who had infrequent sampling or a

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Table I. Plasma fibronectin levels in normotensive gravid patients destined to become preeclamptic

Patient No.	*Plasma fibronectin (μg/ml)*	*Weeks' gestation at time of sampling*	*Days from sample to onset of hypertension*	*Clinical data*					
				Age (yr)	*Race*	*Gravidity and parity*	*Blood pressure (mm Hg)*	*Proteinuria*	*Abnormal edema*
Severe preeclampsia									
1	455	25	70	43	B	G_4P_3	190/100	1+	Pos.
2	615	32.5	7	22	B	G_1P_0	140/90	3+	Neg.
3	630	24	28	23	B	G_2P_1	140/90	3+	Pos.
4	650	30	58	21	B	G_2P_1	150/100	3+	Neg.
	1065	38	8						
Nonsevere preeclampsia									
5	300	34.5	45*	20	W	G_3P_1	150/90	2+	Neg.
6	450	34.5	27	22	B	G_2P_1	140/100	1+	Neg.
7	452	25	106	15	B	G_1P_0	150/80	2+	Pos.
	585	33.5	47						
8	460	16	122	29	W	G_3P_1	150/85	2+	Pos.
9	463	23	129	19	B	G_2P_1	140/85	2+	Pos.
10	470	32	44	20	W	G_6P_0	140/85	2+	Pos.
11	510	33	42	19	B	G_1P_0	150/90	1+	Pos.
12	630	38	30	13	B	G_1P_1	150/100	1+	Pos.
13	645	35	31	24	B	G_1P_0	160/100	3+	Pos.
14	660	38	3	21	W	G_5P_1	150/90	1+	Pos.
15	720	33	51	15	B	G_1P_0	150/100	2+	Pos.
16	750	33.5	14	22	B	G_2P_1	140/90	1+	Neg.
17	840	33	55	24	W	G_1P_1	140/90	Trace	Pos.
Mean ± SD	597 ± 173	30.8 ± 5.7							

*Fibronectin concentration at the onset of labor was 440 μg/ml.

Table II. Plasma fibronectin levels in nonpreeclamptic gravid patients

Patient No.	*Plasma fibronectin (μg/ml)*	*Weeks' gestation at time of sampling*	*Clinical data*					
			Age (yr)	*Race*	*Gravidity and parity*	*Blood pressure (mm hg)*	*Proteinuria*	*Abnormal edema*
1	120	38	20	W	G_1P_0	110/66	Neg.	Neg.
2	200	34	27	W	G_2P_1	110/70	Neg.	Neg.
3	210	38.5	17	B	G_1P_0	120/80	Neg.	Neg.
4	210	32	21	W	G_3P_1	112/82	Neg.	Neg.
5	220	32	23	W	G_4P_2	120/90	1+	Neg.
6	240	34	25	B	G_3P_1	120/70	Neg.	Neg.
7	240	37	21	B	G_5P_4	116/76	Trace	Neg.
8	260	21	21	B	G_2P_1	110/60	Neg.	Neg.
9	265	32	33	B	G_3P_2	110/60	1+	Neg.
10	275	30	23	W	G_2P_1	116/70	Neg.	Neg.
11	280	22	25	B	G_4P_1	106/60	Neg.	Neg.
12	280	37	26	B	G_2P_1	110/80	Neg.	Neg.
13	280	39	23	B	G_3P_0	120/70	Trace	Neg.
14	280	30	27	B	G_2P_1	120/80	Neg.	Neg.
15	285	30	25	B	G_3P_1	120/70	Neg.	Neg.
16	295	39	14	B	G_1P_0	110/70	Neg.	Neg.
17	320	34	24	W	G_2P_1	110/70	Trace	Neg.
18	330	32	23	B	G_5P_2	120/80	1+	Neg.
19	350	32.5	21	W	G_2P_1	130/80	Neg.	Neg.
20	385	28	44	B	G_4P_3	110/60	Neg.	Neg.
21	390	37	28	B	G_7P_4	106/60	Neg.	Neg.
22	395	33	40	W	G_4P_2	110/66	Neg.	Pos.
23	395	30	32	B	G_2P_1	132/80	Neg.	Neg.
Mean ± SD	238 ± 70	32.7 ± 4.8						

short interval between plasma sampling and the onset of hypertension. A single preeclamptic patient (No. 5) had a normal early fibronectin level (300 μg/ml). However, the fibronectin level in this patient was elevated (440 μg/ml) at the time of diagnosis 45 days later, so a rise in the level before the onset of hypertension cannot be ruled out.

In none of the 23 control, uncomplicated pregnancies was an abnormal fibronectin level detected (Table II). The mean (±SD) fibronectin concentration for the control group was 271 ± 70 μg/ml. The difference between the groups was significant ($p < 0.001$).

Comment

These data suggest that the fibronectin rise in preeclampsia may occur before weight gain, abnormal edema, or hypertension becomes apparent. The relationship of the elevation of this glycoprotein to the pathophysiologic processes involved in preeclampsia is not apparent from our study; however, these results do suggest that the rise in fibronectin is not simply a consequence of vasospasm and hypertension characteristic of this disorder. The temporal relationship of the rise in fibronectin levels to the onset of symptoms would suggest that an endothelial cell abnormality may be the earliest pathologic process in these patients, occurring well before hypertension is evident.

Measurement of plasma fibronectin levels would appear to be a sensitive indicator not only for preeclampsia but for diagnosis of incipient preeclampsia in normotensive gravid women destined to become clinically preeclamptic later in pregnancy. Although a larger study is needed to verify the apparent sensitivity, specificity, and predictive value of fibronectin levels found in this small group of patients, these data suggest that further investigation of this glycoprotein might improve neonatal outcome by allowing earlier detection as well as provide insight into the pathophysiologic characteristics of the preeclamptic syndrome.

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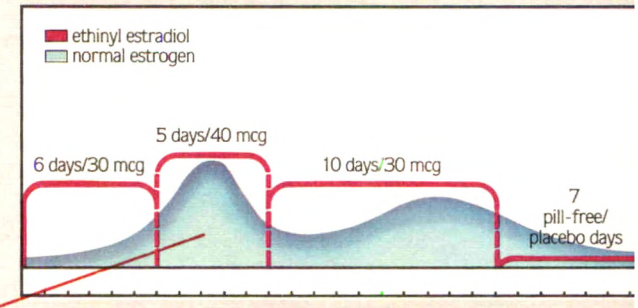
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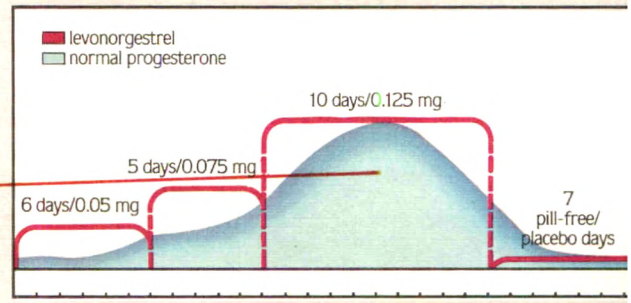
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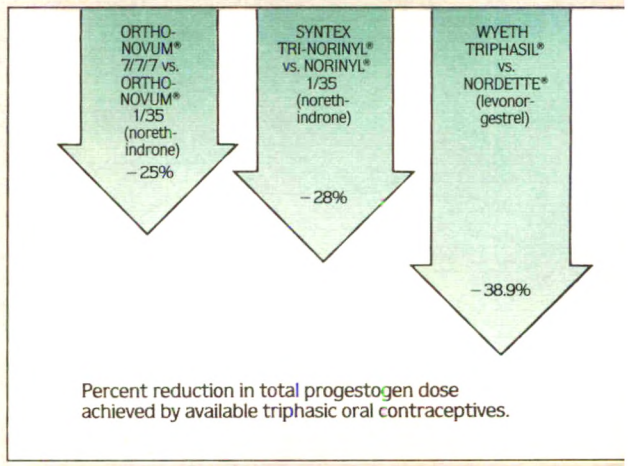
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Percent reduction in total progestogen dose achieved by available triphasic oral contraceptives.

THE TEXTBOOK TRIPHASIC
1986 Edition

NEW STUDIES CORROBORATE INITIAL FINDINGS OF MINIMAL METABOLIC IMPACT

Numerous studies now support the conclusions of initial investigators—that low dose TRIPHASIL® can be expected to have minimal impact on lipid metabolism.

↑ Increased ↓ Decreased ⇌ No Significant Change □ Not Tested

INVESTIGATOR	(MONTHS)	TOTAL CHOLESTEROL	TRIGLYCERIDES	HDL CHOLESTEROL	LDL CHOLESTEROL
GASPARD[1]	6	⇌	⇌	⇌	⇌
VERMEULEN[2]	6	⇌	⇌	⇌	⇌
HARVENGT[3]	6	⇌	⇌	⇌	⇌
SKOUBY[4]	6	⇌	↑	⇌	⇌
MÄRZ[5]	3	⇌	↑	⇌	⇌
BRIGGS[6]	18	⇌	↑	⇌	
CULLBERG[7]	6	⇌	⇌	⇌	
SCHIJF[8]	6	⇌	⇌	⇌	
LARSSON-COHN[9]	6	⇌	↑	⇌	

No significant lipid impact up to 18 months of use

Throughout this extensive battery of studies,[1-9] the TRIPHASIL® formulation produced no significant change in total cholesterol, HDL cholesterol or LDL cholesterol. Some studies did not show a significant increase in triglycerides, while others did. TRIPHASIL® has also been shown to have no significant impact on carbohydrate metabolism[6] or blood pressure means.[10]

No significant impact on BP means or carbohydrate metabolism

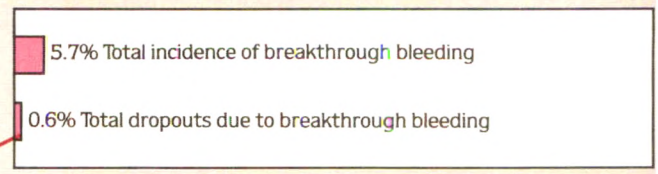
See Wyeth Rep for study details

Triphasil®
Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen
Wyeth
21- and 28-day regimens
A study in documented performance

See important information on last page.

LOW INCIDENCE OF BREAKTHROUGH BLEEDING ENCOURAGES PATIENT ACCEPTANCE

In clinical trials involving 35,036 cycles of TRIPHASIL® use,[10] patients experienced a high degree of contraceptive efficacy and an extremely low incidence of breakthrough bleeding.*



This resulted in an excellent record of patient acceptance. Only 0.6% of patients discontinued their TRIPHASIL® regimen because of breakthrough bleeding.

Only 0.6% dropouts due to BTB

PREFERRED PACKAGING

TRIPHASIL® offers easy-to-remember Day 1 start, with no need for back-up contraception during the first cycle when taken as directed.† The discreet, reusable compact —preferred by patients and physicians[11]—helps patient stay on schedule, month after month.

No need for back-up contraception in 1st cycle

*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

† No first-cycle pregnancies in 3,546 women using no additional protection. In worldwide postmarketing experience, only one first cycle pregnancy has been reported in the literature. (Graham H: Letter to Editor, *Br Med J 284*:422, 1982.) **For dosage and administration, see brief summary on last page.**



Only Triphasil®
Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen
Wyeth
21- and 28-day regimens
A study in low-dose performance

HE TEXTBOOK TRIPHASIC

6 Edition

TRIPHASIL® WORLDWIDE BIBLIOGRAPHY

Performance proven in over 100 million cycles of experience worldwide

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Only Triphasil® Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

Wyeth

21- and 28-day regimens

A study in documented performance

86, Wyeth Laboratories

IN BRIEF:
TRIPHASIL®—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possib types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increas spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended tha any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy i confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation o pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of bec pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clin recommend 3 months, although no precise information is available on which to base this. The administration progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significar percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observe on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three stu were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some wom hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalenc 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previous had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hype sion that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recur persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patie discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional cau should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequat diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while poten useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young w without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinu OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use ot methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularitie

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity a quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk o mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's unt has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in s

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depr recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and us alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with ca monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disc migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be admin with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxin deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containin

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrin induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measu protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, re the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associate concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, p sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral he rhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an a ciation between the following conditions and use of OC's although additional confirmatory studies are needed mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. N and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of p during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. G intestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in n flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of tre edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; c weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactat given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (ste intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the a has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, cl appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema n erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's b young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as d and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of med or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptiv method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

Triphasil®
Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

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Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion

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Control of blood pressure in severe pregnancy-induced hypertension has often relied on agents with an unpredictable onset and duration of action. Because intravenous nitroglycerin is a potent, rapidly acting agent with a hemodynamic half-life measured in minutes, we evaluated its cardiovascular effects with and without volume expansion in six patients with severe pregnancy-induced hypertension. Nitroglycerin alone reduced mean arterial pressure by 27.5% without any significant changes in heart rate, central venous pressure, or stroke volume. The pulmonary capillary wedge pressure fell from 9 ± 3 to 4 ± 2 mm Hg ($p < 0.05$) while the cardiac index decreased from 3.51 ± 0.67 to 2.87 ± 0.76 L/min · m^2. Oxygen delivery fell significantly ($p < 0.05$), from 617 ± 78 to 491 ± 106 ml/min · m^2. While volume expansion alone had no effect on mean arterial pressure, the combination of blood volume expansion and nitroglycerin resulted in a marked resistance to the hypotensive effect of nitroglycerin. Cardiac index, pulmonary capillary wedge pressure, and oxygen utilization were not significantly different from baseline values when volume expansion preceded nitroglycerin. We conclude that the ease with which nitroglycerin reduces blood pressure is dependent on the individual patient's volume status. Although volume expansion allows one to maintain cardiac index, pulmonary capillary wedge pressure, and oxygen utilization when used in combination with nitroglycerin, this benefit may be offset by a concomitant reduction in hypotensive capability. (Am J Obstet Gynecol 1986;154:1053-9.)

Key words: Pregnancy-induced hypertension, nitroglycerin

Pharmacologic control of blood pressure in severe pregnancy-induced hypertension has traditionally relied on the use of hydralazine. While hydralazine is appropriate in most situations, there are some occasions where another agent might be of benefit. One clear circumstance is the patient in whom hydralazine fails to control severe life-threatening hypertension.[1] Another problem area is the patient with severe pregnancy-induced hypertension undergoing cesarean section under general anesthesia. Hydralazine's slow onset of action does not make it an ideal agent for controlling the brief but severe episode of hypertension seen with tracheal intubation.[2] Furthermore, prolonged hypotension secondary to hydralazine in the face of excessive surgical blood loss could compound an already difficult clinical situation.

A less clear but equally important circumstance is the patient with borderline placental function where relatively uncontrolled decrements in blood pressure could adversely affect the fetus.[3, 4] The unpredictability of hydralazine's onset of action as well as duration of action lends itself to this potentially dangerous situation.[5-10]

Intravenous nitroglycerin is a potent, rapidly acting antihypertensive agent with a hemodynamic half-life measured in minutes.[11-13] Although nitroglycerin is thought of primarily as a venodilator, it also induces arterial hypotension.[14-16] These properties make nitroglycerin a candidate for control of the arterial hypertension seen in pregnancy. Because of nitroglycerin's rapid onset as well as ease of regulation, we initiated a prospective study of intravenous nitroglycerin's hemodynamic effects when used for control of blood pressure in severe pregnancy-induced hypertension. In this study we also analyzed the hemodynamic effects of blood volume expansion when used in conjunction with intravenous nitroglycerin.

Methods

Patient population. Six patients with severe pregnancy-induced hypertension, as manifested by a diastolic blood pressure ≥110 mm Hg made up the study group. The mean maternal age was 20.4 ± 2.9 years and the average gestational age was 37.3 ± 3.6 weeks. This study was approved by the Institutional Review

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Table I. Comparison of the effects of nitroglycerin, with and without volume expansion, on heart rate, mean arterial pressure, central venous pressure, pulmonary arterial pressure, and pulmonary capillary wedge pressure

	Baseline	*Nitroglycerin alone*	*Volume expansion alone*	*Volume expansion plus nitroglycerin*
Heart rate (bpm)	74	81	70	85
	104	128	111	111
	111	110	97	122
	86	104	90	96
	95	95	94	108
	100	83	94	103
Mean ± SD	95 ± 13	100 ± 18	93 ± 13	104 ± 13
Mean arterial pressure (mm Hg)	141	115	147	120
	145	105	139	113
	125	102	136	115
	135	121	132	126
	151	103	155	119
	155	76	155	132
Mean ± SD	142 ± 11	103 ± 15*†‡	144 ± 10‡§	121 ± 7*†§
Central venous pressure (mm Hg)	1	2	6	3
	3	0	3	−1
	3	3	5	6
	3	3	1	0
	0	−1	0	5
	3	2	8	2
Mean ± SD	2 ± 1	2 ± 2	4 ± 3	3 ± 3
Mean pulmonary arterial pressure	12	13	22	18
(mm Hg)	17	11	17	12
	12	6	18	10
	13	10	17	12
	12	10	19	13
	15	10	25	14
Mean ± SD	14 ± 2	10 ± 2*†‡	20 ± 3*‡§	13 ± 3†§
Pulmonary capillary wedge pressure	8	8	15	12
(mm Hg)	13	6	13	5
	6	3	13	7
	10	4	11	8
	5	2	10	8
	9	3	20	7
Mean ± SD	9 ± 3	4 ± 2*†‡	14 ± 4*‡§	8 ± 2†§

*$p < 0.05$ versus baseline.
†$p < 0.05$ versus volume expansion.
‡$p < 0.05$ versus volume expansion plus nitroglycerin.
§$p < 0.05$ versus nitroglycerin.

Board for Human Utilization at Baylor College of Medicine.

Hemodynamic measurements. After written informed consent was obtained, percutaneous catheterization of the right side of the heart with a triple lumen, flow-directed pulmonary artery catheter (Swan-Ganz, Edwards Laboratories) and arterial cannulation of the radial artery were performed. The position of the catheter was documented by pressure waveforms and confirmed by a single chest x-ray film.

All measurements were made with the zero reference level at the midaxillary line with the patient supine and a wedge under the right hip. All patients were receiving a continuous intravenous magnesium sulfate infusion of 1.5 to 2.5 gm/hr for at least 1 hour before any measurements were obtained in order to obviate any effects of magnesium sulfate.[17] The following hemodynamic variables were measured: (1) heart rate, (2) mean arterial pressure, (3) central venous pressure, (4) mean pulmonary arterial pressure, (5) pulmonary capillary wedge pressure, and (6) cardiac output. Systemic and pulmonary arterial pressures were determined by waveform analysis with the use of an eight-channel recording system (Hewlett-Packard). Heart rates were derived from an electronic cardiotachometer. Cardiac outputs were determined by a bedside thermodilution cardiac output computer (Hewlett-Packard) with the use of 0° C iced injectate. Five cardiac outputs were performed at each measurement interval. The high and low cardiac output values were discarded and the mean of the remaining three values was recorded. Arterial and mixed venous blood gas samples were obtained anaerobically at the time of each cardiac output determination and analyzed for pH and blood gases

Table II. Comparison of the effects of nitroglycerin, with and without volume expansion, on cardiac index, stroke volume index, left ventricular stroke work index, and systemic vascular resistance

	Baseline	*Nitroglycerin alone*	*Volume expansion alone*	*Volume expansion plus nitroglycerin*
Cardiac index (L/min · m^2)	3.81	3.37	4.02	4.49
	4.26	3.33	4.90	4.08
	3.30	2.33	5.21	4.10
	3.67	3.57	4.40	4.06
	3.74	3.06	4.74	3.80
	2.28	1.60	3.52	3.24
Mean ± SD	3.51 ± 0.67	2.87 ± 0.76*†	4.46 ± 0.61‡§	3.96 ± 0.41§
Stroke volume index	52	42	57	53
(ml/min · m^2)	41	26	44	37
	30	21	54	34
	43	34	49	42
	39	32	50	35
	23	19	37	31
Mean ± SD	38 ± 10	29 ± 9*	49 ± 7§	39 ± 8
Left ventricular stroke work	99	64	109	82
index (gm-m · m^2)	78	37	80	57
	51	30	95	52
	77	58	85	72
	83	47	105	56
	48	20	73	57
Mean ± SD	73 ± 20	43 ± 17*†‡	91 ± 14†§	63 ± 12*§
Systemic vascular resistance index	2941	2675	2800	2094
(dyne-sec/cm^5 · m^2)	2668	2527	2215	2241
	2959	3393	2008	2119
	2873	2633	2381	2483
	3219	2711	2608	2389
	5322	3095	3328	3206
Mean ± SD	3330 ± 992	2839 ± 333	2557 ± 470‡	2422 ± 413‡

*$p < 0.05$ versus volume expansion.
†$p < 0.05$ versus volume expansion plus nitroglycerin.
‡$p < 0.05$ versus baseline.
§$p < 0.05$ versus nitroglycerin.

via an Instrumentation Laboratory 1301 pH/blood gas analyzer. Hemoglobin oxygen saturation and total hemoglobin were measured directly with the use of an Instrumentation Laboratory 282 Co-oximeter System.

Derived variables included cardiac index, stroke volume index, systemic vascular resistance index, and left ventricular stroke work index. All variables were indexed according to the patient's height and weight with the use of a nomogram derived from nonpregnant patients. The body surface has not been determined in pregnant patients, but for comparative reasons standardization was considered necessary. Derived oxygen-related variables measured included the oxygen delivery index and the oxygen consumption index. Standard formulas were used to calculate all derived variables.[18] Analysis of data was by Duncan's multiple comparison technique with the use of the Statistical Package for the Social Sciences; significant levels were taken as $p < 0.05$. Data are shown as the mean ± SD.

Nitroglycerin administration. After a period of at least 1 hour of stabilization, baseline hemodynamic parameters were obtained and intravenous nitroglycerin was initiated at 0.1 μg/kg/min. The nitroglycerin dose was titrated until either maternal or fetal symptoms contraindicated further reduction or a mean arterial pressure of 106 mm Hg was reached. A second set of hemodynamic measurements was then obtained. After the second set of hemodynamics the nitroglycerin infusion was discontinued and each patient underwent volume expansion to at least a pulmonary capillary wedge pressure of 10 mm Hg with either 5% or 25% albumin. Hemodynamic measurements were recorded after volume expansion and then a final set of measurements was obtained after reinstitution of nitroglycerin.

Results

Hemodynamic effects of nitroglycerin. The effects of intravenous nitroglycerin alone, blood volume expansion alone, and nitroglycerin in combination with volume expansion on heart rate, mean arterial pressure, central venous pressure, mean pulmonary arterial pressure, and pulmonary capillary wedge pressure are shown in Table I. It can be seen that nitroglycerin significantly ($p < 0.05$) reduced mean arterial pressure by 27.5% while the mean pulmonary arterial pressure and

Table III. Comparison of the effects of nitroglycerin, with and without volume expansion, on oxygen delivery and oxygen consumption

	Baseline	*Nitroglycerin alone*	*Volume expansion alone*	*Volume expansion plus nitroglycerin*
Oxygen delivery (ml/min · m^2)	585	503	612	661
	732	582	758	670
	610	379	836	661
	628	591	611	559
	652	549	935	580
	494	343	694	550
Mean ± SD	617 ± 78	491 ± 106*†‡	741 ± 129*‡§	614 ± 56†§
Oxygen consumption (ml/min · m^2)	124	104	109	116
	146	147	148	165
	80	78	101	146
	161	115	115	114
	127	144	178	149
	100	78	117	117
Mean ± SD	123 ± 30	111 ± 30	128 ± 29	135 ± 22

*$p < 0.05$ versus baseline.
†$p < 0.05$ versus volume expansion.
‡$p < 0.05$ versus volume expansion plus nitroglycerin.
§$p < 0.05$ versus nitroglycerin.

pulmonary capillary wedge pressure were reduced by 28.6% and 55.6%, respectively. This occurred without any significant changes in heart rate or central venous pressure. The dose of nitroglycerin required to satisfactorily reduce mean arterial pressure was 1.2 ± 0.53 μg/kg/min. In Table II the hemodynamic changes induced by nitroglycerin on cardiac index, stroke volume index, left ventricular stroke work index, and systemic vascular resistance index are shown. Cardiac index and stroke volume index decreased 18.2% and 23.6%, respectively, while the left ventricular stroke work index declined 41.1%. A falling trend in systemic vascular resistance was noted after intravenous nitroglycerin but because of the wide standard deviation this trend was not significant. The lack of change in systemic vascular resistance index was also attributable to the fact that cardiac index fell concurrent with the reduction in mean arterial pressure.

Oxygen-related parameters are detailed in Table III. The oxygen delivery index fell significantly ($p < 0.05$) from 617 ± 78 to 491 ± 106 ml/min · m^2 of body surface area while oxygen consumption decreased from 123 ± 30 to 111 ± 30 ml/min · m^2 (9.8%, NS).

Effects of volume expansion. After the reduction of blood pressure with nitroglycerin alone, the effects of blood volume expansion with either 5% or 25% albumin were studied. Each patient received either 250 ml aliquots of 5% albumin or 50 ml aliquots of 25% albumin every 30 to 40 minutes until the pulmonary capillary wedge pressure was at least 10 mm Hg. The volume of 5% albumin required for four patients was 250, 500, 1250, and 1250 ml, respectively. The dose of 25% albumin was 125 ml in one patient and 100 ml in the other. Intravenous nitroglycerin was then reinstituted with the goal of reducing mean arterial pressure to 106 mm Hg. The effects of blood volume expansion alone are shown in Table I. Volume expansion resulted in significant ($p < 0.05$) increases over baseline values in both pulmonary capillary wedge pressure and cardiac index accompanied by a 23.2% decline in systemic vascular resistance. There was, however, no appreciable effect on mean arterial pressure. Volume expansion increased oxygen delivery from 617 ± 78 to 741 ± 129 ml/min · m^2 ($p < 0.05$). Oxygen consumption did not significantly increase after volume expansion (value before volume expansion was 123 ± 30 ml/min · m^2 and that after volume expansion was 128 ± 29 ml/min · m^2).

Effects of volume expansion and nitroglycerin. In Table I the cardiovascular effects of nitroglycerin with and without blood volume expansion are compared. There was a significantly higher mean arterial pressure after volume expansion. This was due to an inability to lower mean arterial pressure to the range of 106 mm Hg. This level could not be reached even though doses as high as 35 μg/kg/min were given. The pulmonary capillary wedge pressure and cardiac index after nitroglycerin in combination with volume expansion remained significantly ($p < 0.05$) elevated over those values seen with nitroglycerin alone. These indices were not significantly different from baseline values obtained before initiation of any therapy.

The combination of nitroglycerin and volume expansion resulted in an oxygen delivery value of 614 ± 56 ml/min · m^2. This was significantly greater than the 491 ± 106 ml/min · m^2 seen with nitroglycerin alone ($p < 0.05$). Oxygen consumption was 135 ± 22 ml/min · m^2 with combination therapy and 111 ± 30

ml/min · m² with nitroglycerin alone (NS). Although there was a 22% increase in oxygen consumption when combination therapy was compared to nitroglycerin alone this was not statistically significant. Furthermore, combination therapy did not significantly improve oxygen consumption over baseline values obtained before any therapeutic intervention (123 ± 30 ml/min · m²).

Fetal and neonatal outcome. The use of nitroglycerin in these six patients did result in two areas of concern. First, in two patients when nitroglycerin was initiated without volume expansion, the mean arterial pressure was inadvertently and suddenly lowered to <106 mm Hg. Concomitant with the reduction in blood pressure below 106 mm Hg, fetal heart rate abnormalities including late decelerations and bradycardia occurred. In the first patient the mean arterial pressure decreased to 61 mm Hg and returned to 93 mm Hg 13 minutes after discontinuation of the nitroglycerin infusion. Late decelerations began 13 minutes after discontinuation of the nitroglycerin infusion and resolved after 10 minutes. In the second patient, the mean arterial pressure decreased to 76 mm Hg and returned to a mean of 119 mm Hg 1 minute after discontinuation of the nitroglycerin infusion. The fetal heart rate decreased to 75 bpm during the hypotensive episode and returned to 120 bpm 8 minutes after discontinuation of the nitroglycerin infusion. In each case the infusion was restarted at a lower dosing regimen and the ominous fetal heart rate changes did not recur.

The second area of concern was the development of a very flat fetal heart rate tracing with an average beat-to-beat variability of <5 bpm. This occurred in three patients. Because of the absence of any other fetal heart rate changes, labor was allowed to continue. At delivery, umbilical cord gas values were obtained in four of the six infants. These revealed a mean pH of 7.26 ± 0.05 units in the umbilical artery and 7.32 ± 0.03 units in the umbilical vein. The mean Apgar scores for the group at 1 and 5 minutes were 6.8 ± 2.1 and 8.7 ± 0.7, respectively. Five of the six infants had no complications in the nursery and were discharged home with the mother. One infant (the only infant weighing <2300 gm) weighed 820 gm and that infant's hospital course was complicated by respiratory distress syndrome and by intermittent apnea and bradycardia, and prolonged nutritional support was required.

Comment

This study demonstrates that the major cardiovascular effect of intravenous nitroglycerin in patients with severe pregnancy-induced hypertension is effective reduction of the mean arterial pressure as well as the pulmonary capillary wedge pressure and cardiac index. It is of interest that a rapid decline in pulmonary capillary wedge pressure often preceded the expected drop in mean arterial pressure. This observed fall in pulmonary capillary wedge pressure was used as a clinical predictor that the nitroglycerin infusion dose was near the level required to produce hypotension. No significant changes occurred in heart rate, central venous pressure, or stroke volume after nitroglycerin. The decline in cardiac index was also accompanied by a fall in oxygen delivery and consumption. Although we did not evaluate uteroplacental blood flow in this study, the fall in oxygen utilization could conceivably be secondary to this system.

Previous studies in nonpregnant patients have also shown a similar fall in pulmonary capillary wedge pressure with intravenous nitroglycerin.[19-31] The lack of significant changes in heart rate seen in this study is consistent with previous studies of intravenous nitroglycerin.[19, 20, 23-25, 27, 30-33] Other studies have documented either a decrease[22, 26, 29] or an increase[21, 28] in heart rate with nitroglycerin.

A comparison of nitroglycerin's effect on cardiac output with previous studies is difficult as myocardial compromise is almost always present in studies of nonpregnant patients. However, our results are consistent with previous observations that patients with low or normal filling pressures respond to nitroglycerin by a decrease in stroke volume or cardiac output while patients in left ventricular failure cardiac output either maintain or improve cardiac output despite a fall in left ventricular filling pressure.[33, 34] Also Christensson et al.,[19] in a study of intravenous nitroglycerin in normal nonpregnant patients, found a small decrease in cardiac output when patients remained in the supine position and blood pressure remained stable. When continuous breathing against positive pressure was initiated, they documented a 30% fall in stroke volume and cardiac output. The downward trend in cardiac index seen before volume expansion with nitroglycerin was clearly offset after volume expansion. This is consistent with previous studies of nitroglycerin showing an improvement in cardiac output when decreased filling pressures were restored by volume expanders.[35, 36]

The effects of volume expansion alone were an increase in cardiac index and pulmonary capillary wedge pressure without any change in mean arterial pressure. This is not in agreement with previous studies in which volume expansion has resulted in a lowering of blood pressure in severe pregnancy-induced hypertension.[37, 38] We feel that this lack of effect on blood pressure may be attributable to a more severe degree of hypertension in our study population. Additionally, the increase in cardiac output most likely offset any reduction in compensatory vasoconstriction seen because of hypovolemia.

The combination of nitroglycerin and volume expansion allowed a reduction in mean arterial pressure

while cardiac index and oxygen utilization were maintained in the range seen before therapy. However, there was no increase in these parameters compared with pretherapy values and volume expansion prevented lowering of the mean arterial pressure to levels obtained with nitroglycerin alone. This resistance to intravenous nitroglycerin was seen despite a greater than tenfold increase in the dose of nitroglycerin.

The occurrence of diminished fetal heart rate variability in three of the five patients in this study is of interest. A possible explanation is the observation that a loss of cerebral autoregulation and increased intracranial pressure occur in cats[39] as well as humans[40-42] after nitroglycerin. This may be due to increased capacitance vessel dilatation and increased cerebral volume. Although there appeared to be no adverse effects on fetal acid-base status in this study, the documentation of a decrease in oxygen delivery when nitroglycerin was instituted in a non–volume-expanded state is worrisome. Further investigation with an expanded patient population and neurobehavioral testing of the neonate appear necessary before nitroglycerin is recommended under less than life-threatening circumstances.

In summary, intravenous nitroglycerin is an efficacious agent for the reduction of blood pressure in the pregnancy complicated by hypertension. It appears that the ease with which blood pressure can be reduced is dependent on the volume status of the individual patient. In patients with a relatively low pulmonary capillary wedge pressure an easily obtainable decline in mean arterial pressure can be achieved. This fall in mean arterial pressure is also accompanied by a fall in cardiac index and oxygen utilization. The combination of blood volume expansion and nitroglycerin does allow cardiac index and oxygen utilization to be maintained at higher levels than those seen when volume expansion is not used. However, this benefit may be offset by a concomitant reduction in nitroglycerin's hypotensive capability. Finally, a decrease in fetal heart rate variabilty does occur in some patients after institution of nitroglycerin. Further work with an expanded study population is needed to establish whether this occurrence adversely affects neonatal outcome.

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A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes

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To address the issues of whether corticosteroid treatment and prolongation of the latent phase improve the outcome of pregnancy in patients with preterm premature rupture of the membranes, we studied 96 patients with premature rupture of the membranes who were delivered of infants of adequate weight for gestational age with birth weights between 751 and 2000 gm and who had a latent period longer than 48 hours. Of these 96 infants, 53 received treatment with steroids and tocolytic agents and 43 received no treatment. We found a significant decrease in perinatal mortality and in the incidence of moderate and severe hyaline membrane disease in infants whose mothers received glucocorticoids. The protective effect of glucocorticoid therapy was limited to infants with a birth weight between 751 and 1000 gm or with a gestational age of 27 to 28 weeks. We also found a significant increase in perinatal mortality, mainly due to infection, when the latent phase was prolonged for >7 days, regardless of the type of management. (AM J OBSTET GYNECOL 1986;154:1059-63.)

Key words: Fetal membranes, premature rupture, corticosteroids, hyaline membrane disease

There are several controversial areas in the management of patients with preterm premature rupture of the membranes. Significant discrepancies in opinion involve the maternal and fetal advantages and disadvantages of prolongation of the latent period and the use of glucocorticoids to stimulate fetal lung maturation. This controversy has resulted in different approaches to the management of these patients and led us to study retrospectively the outcome of patients with preterm premature rupture of the membranes man-

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Table I. Comparison between steroid-treated and untreated patients

Characteristic	*No treatment*	*Steroid-treated*
Infants (n)	43	53
Race		
White	22	27
Black	21	26
Primigravid (n)	10	10
Latent phase (days)		
Mean ± SD	5.97 ± 5.51	5.65 ± 4.99
Range	2 to 21	2 to 27
Gestational age at delivery (wk)		
Mean ± SD	30.05 ± 2.6	30.08 ± 2.2
Range	26 to 35	26 to 35
Birth weight (gm)		
Mean ± SD	1417 ± 37	1398 ± 302
Range	790 to 1990	850 to 1900
Sex		
Male	20	29
Female	23	24
Apgar score <6		
At 1 min	25 (58%)	31 (58%)
At 5 min	13 (30%)	16 (30%)

aged with or without steroid administration. We attempted to determine whether significant maternal or fetal risks were associated with prolongation of the latent period and if maternal glucocorticoid therapy offered significant advantages in the management of the problem.

Material and methods

We collected information about the outcome of pregnancy in 439 patients (approximately 4% of all the obstetric admissions to the hospital during the study period) who had an admitting diagnosis of preterm premature rupture of the membranes. The diagnosis of ruptured membranes was accepted if there was documentation of appropriate color changes in Nitrazine paper and the presence of ferning on microscopic examination of the dried vaginal fluid obtained by a sterile speculum examination. The diagnosis of ruptured membranes was confirmed by a real-time ultrasound demonstration of reduced or absent amniotic fluid in the uterine cavity.

At the time of the data review, we excluded from analysis all patients who were delivered of babies with birth weights >2000 gm or with a gestational age longer than 34 weeks. Also excluded were all patients delivered of babies with birth weights <751 gm or with a gestational age shorter than 26 weeks, all infants who were products of twin gestation and who remained in an intact amniotic sac, and all infants classified as small for gestational age by the pediatrician. Patients with diabetes, chronic hypertension, or preeclampsia complicating their pregnancies were excluded, as were patients with a latent phase (interval between time of rupture and time of delivery) of <48 hours. We also excluded all patients who received incomplete treatment with glucocorticoids and those who did not receive corticosteroids but were treated with labor-inhibiting drugs. Also excluded were patients with a positive shake test or phosphatidylglycerol determinations in amniotic fluid specimens collected from vaginal pools.

After all exclusions, 96 patients remained: 43 received no treatment, and 53 were treated with glucocorticoids and β-sympathomimetics (betamethasone, 12 mg intramuscularly, in two consecutive doses 24 hours apart, and terbutaline, 2.5 mg orally, every 4 to 6 hours for a minimum of 72 hours). These 96 patients were delivered of their infants when labor occurred, when chorioamnionitis was diagnosed or suspected, and when there was evidence of fetal distress. Obstetric indications determined the route of delivery. All babies in breech presentation were delivered by cesarean section. Prophylactic antibiotics were given, after cord clamping, to patients who were delivered by cesarean section and after vaginal delivery to patients with obvious signs and symptoms of chorioamnionitis.

At delivery the newborn infants were examined by the pediatricians, had surface cultures done, and were transferred to the appropriate nursery. Cultures were obtained from multiple sites in all neonates. The diagnosis of neonatal sepsis was reached if there was a positive culture from blood, urine, or cerebrospinal fluid. The gestational age was assigned after physical and neurological examination according to the method of Dubowitz et al.[1] The Apgar scores were assigned by the pediatrician attending the delivery.

Chorioamnionitis was a presumed diagnosis based on fetal or maternal tachycardia, elevated maternal leukocyte count with a shift to immature forms, elevated maternal temperature (≥100.6° F), or foul-smelling vaginal discharge. A positive amniotic fluid or cervical culture was not required for this diagnosis. The diagnosis of endometritis was a clinical diagnosis based on the findings of fever, a boggy and tender uterus on abdominal examination, and foul-smelling lochia, regardless of the results of the cervical or endometrial cultures. Febrile morbidity was defined as a temperature of ≥99.5° F on at least two occasions at least 24 hours apart.

The diagnosis of hyaline membrane disease was made if there were clinical symptoms (tachypnea, hypoxia) and typical findings on chest X-ray examination. For the purpose of this review, hyaline membrane disease was classified as mild (no need for positive-pressure ventilation; oxygen for up to 24 hours), moderate (positive-pressure ventilation for <24 hours; oxygen for >24 hours), and severe (positive-pressure ventilation and oxygen for >24 hours). Transient tachypnea of the newborn and neonatal pneumonia were separated from hyaline membrane disease. Intraventricular

Table II. Perinatal mortality by treatment and birth weight

	Birth weight						*Total*	
	751-1000 gm		*1001-1500 gm*		*1501-2000 gm*			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
No treatment	6/9	66.6	3/12	26	3/22	13.5	12/43	27.9
	p = 0.036		NS		NS		p = 0.035	
Treatment	1/8	12.5	4/22	18.18	1/23	14.3	6/53	11.3

Table III. Perinatal mortality by treatment and length of the latent phase

	Length of latent phase					
	>48 <72 hr		*>3 <7 days*		*> 7 days*	
	n	*%*	*n*	*%*	*n*	*%*
No treatment	6/13	7.6	4/18	22.2	7/12	58.3
	p = 0.01		NS		NS	
Treatment	0/12	0	2/29	6.8	4/12	33.3

hemorrhage was diagnosed by computerized tomographic scanning or by ultrasound examination. Intraventricular hemorrhage was classified as grade I to III according to Volpe.[2] Neonatal morbidity and mortality were assessed and recorded up to the time that the infants were discharged from the hospital.

The analysis of the data was carried out by means of two-tailed *t* test and Fischer exact probability test. All computations were made with the SAS version 5 statistical software.

Results

Characteristics of the patients. Given the retrospective nature of this study, it was surprising not to find significant differences between treated and untreated patients in terms of racial composition, number of primiparous patients, and duration of the latent period before admission. Similarly, no significant differences were found with respect to gestational age, birth weight, sex, and number of infants with low Apgar score at birth (Table I).

Maternal outcome. Maternal outcome was similar for the two groups of patients, and the incidence of cesarean section deliveries, chorioamnionitis, endometritis, and febrile morbidity was not significantly different between treated and untreated patients.

Fetal outcome. There was a significant difference in perinatal mortality between treated and untreated patients. In fact, there were six deaths among the 53 patients treated with steroids as compared with 12 deaths in the 43 patients who did not receive steroids ($p < 0.01$). Table II shows that the decrease in perinatal mortality observed among steroid-treated patients was significant only in babies with birth weights between 751 and 1000 gm. Table III shows that the perinatal mortality in both steroid-treated and untreated patients increased with prolongation of the latent phase. Table III also shows that the protective effect of steroid treatment was statistically significant only for patients with a latent phase >2 but <3 days in duration.

Table IV shows the incidence of culture-proved sepsis in steroid-treated and untreated neonates in relation to the length of the latent phase. Neonatal sepsis increased with prolongation of the latent phase for >7 days. In fact, sepsis was responsible for seven of 11 (63.6%) perinatal deaths occurring in patients who remained undelivered for more than 7 days after premature rupture of the membranes. The most frequently isolated pathogens in patients with neonatal sepsis were group B β-hemolytic streptococcus (four cases) and *Escherichia coli* (three cases).

Table V shows that the incidence of moderate and severe hyaline membrane disease was significantly less in steroid-treated patients than in untreated patients both in general and for infants with a birth weight between 751 and 1500 gm.

Comment

The combination of ruptured membranes and preterm gestation is a major cause of perinatal morbidity and mortality because of the high incidence of hyaline membrane disease, intraventricular bleeding, and neonatal sepsis. There is considerable controversy in the literature about the effect of prolonging the latent phase on the incidence of hyaline membrane disease. A recent excellent review[3] found 11 articles showing no difference in hyaline membrane disease with prolongation of the latent phase and 14 showing a decreased incidence. Although it is generally accepted that maternal treatment with corticosteroids reduces

Table IV. Neonatal sepsis by treatment and length of the latent phase

	Length of latent phase		
	>48 <72 hr	*>3 <7 days*	*>7 days*
No treatment	3/13	1/18	3/12 (3)
	NS	NS	NS
Treatment	0/12	2/29 (1)	5/12 (4)
Total	3/25	3/47 (1)	8/24 (7)

Numbers in parentheses are neonatal deaths due to sepsis.

Table V. Moderate and severe hyaline membrane disease by treatment and birth weight

	Birthweight						*Total*	
	751-1000 gm		*1001-1500 gm*		*1501-2000 gm*			
	n	%	*n*	%	*n*	%	*n*	%
No treatment	7/9	77.7	3/12	25.0	2/22	9.0	12/43	27.9
	NS		NS		NS		p = 0.035	
Treatment	3/8	37.5	12/22	9.0	1/23	4.3	6/53	11.3

the incidence of hyaline membrane disease in preterm gestations with intact membranes,[4] the effectiveness of this treatment in reducing the incidence or severity of hyaline membrane disease in patients with preterm premature rupture of the membranes is also controversial. Of nine articles in the recent literature that addressed this issue, four[5-8] suggest a positive effect and six[9-14] do not. In perhaps the best-designed study, Garite et al.[12] managed a group of 80 patients treated with glucocorticoids and compared their outcome with that of a similar number of untreated control patients and found no significant difference in the incidence of respiratory distress syndrome at different gestational ages. Among their least mature neonates (28 to 30 weeks), treated infants had a 25% incidence of respiratory distress syndrome compared with 48% in their control patients. Because of the small number of infants in this gestational age category, no statistical significance was established. They concluded that corticosteroids were not beneficial in patients with premature rupture of the membranes and recommended against their use. However, their two treatment regimens were not completely comparable because the steroid-treated patients were actively delivered after 48 hours and the untreated patients awaited delivery for obstetric reasons. Another prospective comparison[13] reached a similar conclusion but the duration of the latent phase in their steroid-treated and expectantly managed patients was significantly different (39.5 ± 12.8 hours in the steroid-treated group versus 94.6 ± 12.9 hours in the expectant management group).

A recent retrospective review[14] also denies the beneficial effects of glucocorticoid treatment in patients with preterm premature rupture of the membranes. However, although not stated in the article, the two treatment groups seem to be significantly different with respect to the duration of the latent phase (122.8 ± 237.8 hours in the control group versus 55.2 ± 13.7 hours in the glucocorticoid group) because steroid-treated patients were "timely" delivered.

In this study we found that management of patients with preterm premature rupture of the membranes including glucocorticoid administration, tocolysis with terbutaline, and prolongation of the latent phase was more advantageous than prolongation of the latent phase alone. The advantages were a decreased perinatal mortality and a decreased incidence of moderate and severe hyaline membrane disease. The beneficial effects of steroid and tocolysis administration were limited to pregnancies with babies between 751 and 1000 gm and with latent phases lasting >2 but <7 days. However, the value of these findings is limited by the retrospective nature of the study and the lack of adequate randomization of patients between treated and untreated groups.

Our results contradict the observations of Garite et al.,[12] Nelson et al.,[13] and Iams and Barrows.[14] We believe that the different results can be explained by the duration of the latent phase in steroid-treated and untreated patients, which is similar in the present study but was different in the work of Nelson et al.[13] and Iams and Burrows.[14] Also the duration of the latent phase in patients receiving steroids in this work (5.97 ± 5.5 days) seems to be significantly greater than in the patients of Garite et al.[12] (2.73 ± 1.7 days) because their treated patients were actively delivered after 48 hours while ours waited for the spontaneous onset of labor. Also all of our steroid-treated patients and none of the

untreated patients received terbutaline. β-Adrenergic agents may have an effect on fetal lung maturity and help to prevent the development of hyaline membrane disease,[15] and this is an additional factor that may partially account for the differences between our results and those of other studies.

The lack of timed delivery in the management of our patients made it possible to compare the neonatal outcome of some steroid-treated and untreated patients who remained undelivered >7 days after ruptured membranes. Our findings in this subgroup of patients suggest that the advantages of prolongation of pregnancy and treatment with steroids and tocolytic agents are lost after a latent phase of ≥7 days because of the increased incidence and severity of neonatal sepsis. This is an observation that, if supported by prospective studies, may indicate the need to deliver patients with preterm premature rupture of the membranes when they are close to a latent phase of 7 days.

In summary, the data collected in this study support the hypothesis that seroids, tocolysis, and prolongation of the latent phase are beneficial to patients with preterm premature rupture of the membranes when the birth weight is between 751 and 1000 gm or the gestational age is 27 to 28 weeks. The controversy in this area, however, has not concluded, and prospective studies using protocols whereby treated and untreated patients have a similar length of the latent phase are necessary and urgent.

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Reported sexual assault in Nashville–Davidson County, Tennessee, 1980 to 1982

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A review of the experience of 440 women reporting they had been raped was made from their initial contact with the police, through their medical-forensic examination at the county hospital emergency room, by any judicial proceedings, and by their longer term psychological counseling. The data were then compared with those from other municipalities. The assailant's use of a weapon seemed the strongest variable correlated with the victim's decision to press for prosecution. Being raped by a stranger or being physically injured did not correlate with this decision. Women most likely to have been victims of a previous rape were over 40 years old, were black, were assaulted by someone well known to them, or had some sort of mental health problem, and these variables were independent of each other. This implies these victims somehow suffer from an increased vulnerability to repeated rape. The data suggest that use of a county hospital emergency room for the medical management of rape victims discourages their reporting the event to the authorities and their seeking medical treatment. (Am J Obstet Gynecol 1986;154:1064-8.)

Key words: Reported sexual assault, sexual abuse, rape

The sexual abuse of women and children is an ancient problem, yet recently there has been an increase of the public's awareness of this issue. Several reports have appeared on the experiences of various municipalities concerning this problem.[1-5] This report provides this information for Nashville, Tennessee, and provides analysis of the many variables involved.

During the period of this study, the Metropolitan Nashville General Hospital served Davidson County in providing organized emergency health care to victims of sexual assault. The police brought all victims to the emergency room for examination and treatment. Individuals who arrived at the emergency room to first report the incident were given medical treatment and encouraged to report the episode to the police. Other health workers were involved with more long-term mental health issues. The experience then at the hospital was that for the entire county regarding emergency care for victims reporting a recent sexual assault. The unreported activities of private doctors and the unreported experiences of victims are unknown.

When an adult victim arrived in the emergency room, she was examined by a gynecology house officer who treated any injuries, collected documentation and forensic specimens using a standard rape evaluation form and rape kit, and provided prophylaxis against venereal disease and pregnancy. The Victim Intervention Program of the Metropolitan Police Department provided trained crisis intervention personnel to aid the victim during the immediate crisis and to act as a witness advocate during any court proceedings. Mental health situations requiring intense psychiatric intervention were identified by these personnel and referrals were made.

Cases involving children tended to be more chronic in nature and were usually managed through the pediatric clinic. These cases are not included in this study. Acute pediatric cases in which the child was seen in the emergency room are included but represent only a small fraction of the reported child sexual abuse cases.

When an arrest was made, the prosecution of the suspect was managed by a special division of the District Attorney's office. The forensic specimens were processed by the Tennessee Bureau of Investigation (TBI).

Material and methods

From July, 1980, through June, 1982, each patient seen in the Metropolitan Nashville General Hospital who reported having been sexually assaulted was included in this retrospective study. Data were collected from a review of the hospital record and then, for each patient, from personnel interviews and/or file reviews from the police department, the District Attorney's office, and the TBI. Cases involving arrest and trial without the victim visiting the emergency room and rape-homicide cases are not included.

Individual members of the Sexual Assault Study Group and their positions or affiliations are listed at the end of the article.

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Demographic data for the victim came from all available sources. When interviewed, the victim was asked if she had ever been raped before; a positive response was not broken down into childhood sexual abuse or a similar adult assault.

A wet preparation taken from the posterior fornix and a permanent stained slide of similar material was immediately examined by the gynecologist. The presence of motile-nonmotile sperm was recorded. The permanent slide was also examined by the TBI. A positive smear was one with whole or fragmented sperm recorded by either the gynecologist or the TBI. Vaginal and salivary specimens were collected and assessed for the presence of vaginal acid phosphatase and the secretory antigens of the ABO system.

Some of our tables present data as "adjusted frequency percent." This means that for each variable patients with missing data were excluded from the analysis, and the percent is of the total number with valid data. The number of valid cases for each variable is presented along with the adjusted frequency percent. Data were evaluated by means of cross-tabulations and logistic regression. Tests of significance by χ^2 were not appropriate since the data base represented the totality of the population studied and conclusions were not based on analysis of a representative sample.

Results

During the study, 500 people were seen who reported having been sexually assaulted. Of these, 17 were male, all of whom were either children or incarcerated or mentally retarded adults. Five women refused examination and 26 (5%) left against advice before being seen by a physician. There were 12 cases (2%) where the circumstances were such that the report was deemed unfounded by the police and the medical examination not requested. A total then of 440 women of all ages are included in this study. Not all victims were sure if vaginal, oral, or anal penetration had occurred. Twenty-eight thought that it had not, and thus the attack would seem not to meet the legal definition of rape, which requires that some sort of penetration (carnal knowledge) occur. While none of these 28 women had sperm in the vagina, three had objective evidence of vulvar trauma.

Table I presents demographic data for the victims. Overall, 49% of the victims said the assailant was a total stranger, while 20% said he was well known to them as an ex-husband-boyfriend, employer, neighbor, or relative. For 31%, he was known to her more casually as a friend of a friend, previous superficial acquaintance, or someone recently met at a party, bar, or the like. Victims less than 10 years old tended to know their assailants well. Black victims 21 to 30 years old were less likely to be raped by a total stranger than white victims of the same age (47% versus 69%). Victims at both extremes of age were likely to be assaulted in their own homes.

Table I. Demographic data for victims

	Adjusted frequency %
Age (yr) (n = 439)	
<10	6.8
10-15	15.9
16-20	26.9
21-30	35.5
31-40	8.7
41-50	3.0
>50	3.2
Race (n = 439)	
Black	37.4
White	62.0
Other	0.6
Marital status (n = 425)	
Single	74.6
Married	14.1
Separated	1.4
Divorced	8.0
Widowed	1.9
Contraceptive practice (n = 440)	
None	43.9
Oral contraceptives	15.5
Intrauterine contraceptive device	4.5
Barrier	3.3
Tubal ligation	7.2
Other*	25.5
Pregnant at time of assault	3.8
History of previous assault (n = 395)	20.0
History of mental illness† (n = 390)	27.9

Adjusted frequency is the percent of the total group with valid data for that variable (see text).

*Prepubertal, postmenopausal, hysterectomy, partner with vasectomy.

†Diagnosed psychiatric disorders, alcoholism, substance abuse, mental retardation.

About one in every five victims reported that she had been sexually assaulted before. Of the recurrent assault victims aged ≥16, 23% were assaulted by someone well known to them, while only 1% of first-time victims knew their assailant well. Victims aged ≥16 knew their assailant well 13% of the time and 28% of these had been previous victims of sexual assault. Of those assaulted by a stranger, only 12% had been previous victims.

The rates per capita were similar for both races in that younger women seem more likely to be the victims of rape. Black subjects, however, showed a persistently higher per capita rate than white subjects for all ages (Table II).

For victims 16 years of age or older, almost 50% were single, and 51% were vulnerable to pregnancy on the basis of the type of contraception used for voluntary coitus. This did not take into account the victim's menstrual phase or whether sperm were found in the va-

Table II. Reported rapes of women by age and by race-specific rates per thousand per year, 1980 to 1982

Age (yr)	*Black*	*White*
10-15	2.9 (15)	1.7 (20)
16-20	3.65 (22)	2.65 (37)
21-30	2.4 (31)	1.25 (47)
31-40	0.7 (5)	0.5 (13)
41-50	0.1 (1)	0.3 (6)
Over 51	0.15 (2)	0.1 (5)

Absolute numbers of victims are enclosed in parentheses. Children <10 years old were excluded.

gina. Pregnancy prophylaxis was discussed with all victims, but when all things were taken into consideration prophylaxis was given to only 10 patients. There were two victims in the study who probably did become pregnant as a result of the rape, and both had refused pregnancy prophylaxis.

Assault frequencies were independent of the season of the year, but 72% of the attacks occurred between 6:00 PM and 6:00 AM. Table III presents demographic data concerning the assault event itself. Those occurring at the assailant's home usually involved an acquaintance and the victim voluntarily accompanied the assailant to his home. Victims 16 to 30 years old were assaulted less frequently in their own home or their assailant's home. There was one assailant in 84% of the cases, two assailants in 10%, and more than two in 6%.

A weapon, most commonly the handgun, was involved in 39% of the cases. Weapon use was not related to the time of day or year or to the race of the victim. Weapons were rarely involved when the victim was less than 15 years old. Victims aged 21 to 30 had weapons used against them more often (52%) than those aged 16 to 20 (35%) or over 31 (45%). The age of the victim, however, did not correlate with which type of weapon was used. Assaults involving a knife or club were associated with about twice the rate of nongenital victim injury as cases involving a gun (61% and 79% versus 34%).

Victim injury was not more likely if multiple assailants were involved. Most physical injuries consisted of abrasions, scratches, or bruises that required little or no treatment. Genital injuries were those apparent without the use of special magnification or staining. Five patients were hospitalized because of physical injury; four of these were for neurosurgical indications, and in one patient a posterior vaginal fornix laceration required operative repair.

Forced penile penetration was reported to have occurred by 384 of the victims: 63% vaginally only, 2% orally only, 1% anally only; the remainder involved a combination of these. Before coming to the hospital 6% of the victims douched, 20% took a bath, and 26% changed their clothing. One third of the victims arrived at the hospital within 1 hour of the assault and 81% within 4 hours.

Table III. Demographic data for the assault event, given in adjusted frequency percent

	Adjusted frequency %
Location of assault (n = 418)	
Victim's home	26.3
Assailant's home	15.1
Automobile	18.2
Other*	40.4
Weapon use (n = 418)	
Gun	19.4
Knife	15.1
Club	1.0
Other	3.6
None	60.9
Victim injured (n = 430)	
Nongenital, treatment needed	0.9
Nongenital, no treatment needed	40.0
No nongenigal injury	59.1
Genital, treatment needed	0.2
Genital, no treatment needed	16.1
No genital injury	83.7
Hours from assault until hospital visit (n = 352)	
<1	33.2
1-2	21.9
3-5	30.7
6-12	12.5
13-24	1.5
>24	0.2

*Public park, field, vacant lot or building.

Venereal disease prophylaxis was discussed with all patients and given to 86%. Endocervical cultures were positive for *Neisseria gonorrhoeae* in 6.5%. Of the rectal cultures taken, 6.7% were positive, as were 2% of the pharyngeal cultures. There were 362 serologic tests for syphilis done at the initial visit, and 1.7% were positive.

Motile sperm were present in the wet preparation from the vagina in 17% of the victims. In 26%, nonmotile sperm were seen. In only two cases were sperm identified on the wet preparation while the permanent stain was read as negative. There were 44 cases where the wet preparation was negative and the permanent stain positive.

While in the emergency room, 69% of the victims expressed a desire to pursue prosecution if a suspect could be arrested. Of these, 10% later changed their mind and dropped charges. The individual reasons the victim had for not wishing to prosecute did not correlate with age or race. Neither was her knowing the assailant or sustaining injury during the assault related to this decision. If a weapon was used, however, the victim desired prosecution more often (79% versus 55%). The age and race of the victim did not alter her

likelihood to change her mind and later drop charges; neither did her relation to the assailant or his use of a weapon.

Of the 243 victims who persistently desired to prosecute, a suspect was arrested in 132 of the cases. Thus for 46% prosecution was desired but a suspect was never arrested. If an arrest was made, 53% occurred within 24 hours after the assault, 70% by the first week. A suspect was arrested more often if the victim was a child, knew her assailant, or was physically injured during the assault. The frequency with which an arrest was made did not correlate with the victim's age, race, or marital status or with her having a history of a mental health problem.

Only 16% of the victims ever returned to the gynecology clinic for a follow-up visit. The personnel at the Victim Intervention Program, however, were able to contact and follow up on at least 85% of the cases. In 63% they met with the victim on multiple occasions. Of the 249 women with follow-up data available, 37% suffered significant psychiatric complications warranting referral for more intensive psychotherapy. Those women who did develop signifcant psychiatric complications were more likely to have a previous history of a mental health problem (71% versus 19%).

Comment

These data came from women who chose to report the assault. Since many sexual assaults are probably not reported,[6] one may not extrapolate conclusions from this study to all rape events as a whole. For example, the data suggest black women are more likely per capita than white women to sustain a sexual assault. This could result from more black women per capita being assaulted or from reluctance of white victims to report the event and be seen in the county hospital.

On comparison of the data concerning rape in Memphis, Tennessee, reported by Solola et al.[2] and the State Center for Health Statistics,[7] one finds that in Memphis 3.5 per 1000 women aged 16 to 30 reported being raped in 1980, while in Nashville that figure was 1.9 per 1000. Is this higher per capita rate simply because black women seem more likely to be raped and Memphis has a larger percentage of blacks? If the racial distribution of 16- to 30-year-old women in Memphis were the same as that in Nashville, then with the use of demographic standardization the overall rate in Memphis would be 2.1 per 1000. However, this figure was 3.5 per 1000, suggesting some other factor must be involved. Another approach to this question is to compare the per capita rates for black women in the two cities. For 1980, 1.4 per 1000 black women of all ages reported being raped in Nashville, while in Memphis that figure was 2.4 per 1000. Futhermore, in Nashville 0.7 per 1000 white women of all ages reported rape in 1980, while in Memphis this number was 0.9 per 1000. Thus for both races a larger number per capita reported being raped in Memphis. This could be because male citizens of Memphis are more prone to committing rape than those of Nashville. It seems likely however, that the use of a noninstitutionalized rape center in Memphis is more encouraging for victims to report the event and seek treatment.

Comparing other characteristics of the assault event in Nashville with those for Memphis[2] and other cities,[1, 3-5] one finds consistency in the reported distribution of the victim's age, the lcoation of the assault, the use of a weapon and the type of weapon, the relation of the victim to the assailant, the number of cases involving multiple assailants, the site(s) of forced penetration during the assault, and the most frequent time of day. Nashville differed from Memphis in that 41% of our victims suffered some form of nongenital injury, while only 23% were so injured in Memphis. This difference may be because rape in Memphis is less violent than in Nashville but again is probably because a victim without physical injury is more likely to report the event and seek treatment if she does not have to go to a county hospital emergency room for care.

Although one in every five patients in our series reported having been victim of a previous sexual assault, 66% of these women aged 16 or older also had a history of some sort of mental illness. This correlation was not because "crazy people make up stories"; the percent of victims with objective evidence of violence (injury) and penetration (sperm in the vagina not accounted for by voluntary coitus) was the same whether or not the victim had a history of mental illness. These individuals are more vulnerable to repeated sexual abuse perhaps because of their housing arrangements, because they have an impaired judgment of character, and/or because they have decreased defense capacities. Along this line, almost one fourth of recurrent assault victims were assaulted by someone well known to them whereas only 1% of first-time victims knew their assailant well. Again, recurrent assault victims seem somehow to suffer from an increased vulnerability. Women over 40 years old and black women were also more likely to have been victims of a previous sexual assault. This correlation persisted after exclusion of those with a history of a mental health problem and could not be accounted for simply by compounding the chance of a single random event as the patient aged or simply because black women have an overall higher per capita assault rate than white women and would therefore be more likely to be assaulted more than once. It may be that older women and black women are more likely to live alone and are perceived as easier targets by the rapist.

When reporting on a similar series from Toronto in 1977, Clark and Lewis[8] stated that women who were

young, were single or divorced, were on welfare, or had a history of a mental health problem were unlikely to have the police follow through with the case and make an arrest. They concluded that the police did not take the case seriously and they categorized these victims as "women who cannot be raped." The data from Nashville in the 1980s do not repeat this finding. The likelihood that an arrest was made for cases where the victim desired to press for prosecution was not influenced by her age, race, being single or divorced, or having a history of a mental health problem. This may be because a Sexual Crimes Unit that was established by the Police Department was specially trained and sensitized to the issues involved in sexual assaults. Victims who were children or who knew their assailant casually or well were more likely to have a suspect arrested than those for whom the assailant was a total stranger. This was probably because these assailants were easier to locate and identify. The other factor that did correlate with the likelihood of arrest was whether the victim sustained nongenital injury. Victims who were "beaten up" and desired prosecution were more likely to have an arrest made than those without this objective evidence of violence (52% versus 35%), and this was independent of whether the victim knew her assailant.

Only 59% of these victims persistently desired to prosecute a suspect if he could be arrested. Most commonly, the victim was reluctant to become involved in long court proceedings and the attendant publicity involved. This reluctance was similar despite the victim's age or race or whether the assailant was a total stranger or someone she knew. Even victims who were beaten were no more likely to desire prosecution than those without such injury. One variable that did correlate with the victim's desire to prosecute was the assailant's use of a weapon. In such cases, especially in those more than 30 years old, the victims were 24 percentage points more likely to press for prosecution than when no weapon was involved. Why weapons overcame the victim's reluctance to prosecute may be related to her feeling an intense trauma, a deeper sense of fear she would be murdered, and a more profound anger than when the assailant used no weapon. His use of a weapon could also deepen the victim's sense that she was not at fault, that the assailant's motives were corrupt from the start, and that more people would believe her claim that she was in fact raped.

The plight of the victim during court proceedings has been popularized, but this has also spread the unwanted result of women feeling that any attempt to bring the rapist to justice is useless and doomed to failure. There may be many good reasons a victim chooses not to prosecute, but a defeatist attitude does not serve the needs of women. In Nashville, special services are available to victims through the criminal justice system and the Rape and Sexual Abuse Center to assist and encourage those who choose to prosecute their assailant. Hopefully, this support will help overcome the sense of shame and helplessness felt by victims of rape and will help law enforcement agencies apprehend rapists and bring them to justice.

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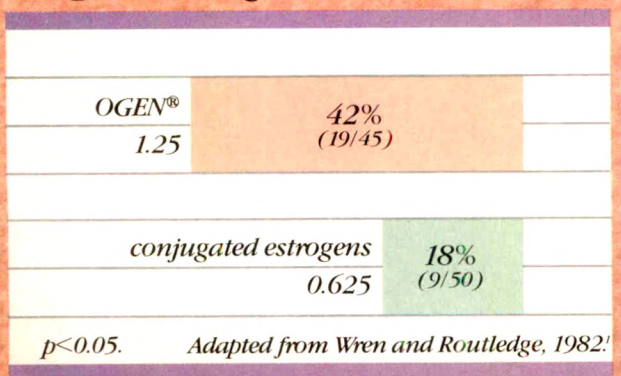
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See adjacent page for brief summary of prescribing information.

Reference:
1. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

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OGEN®

ESTROPIPATE TABLETS, USP

Tablets

WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of *broad spectrum antibiotics* which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving *insulin* may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Abnormal glucose tolerance test results.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Change in cervical eversion and in degree of cervical secretion.
Breakthrough bleeding, spotting, change in menstrual flow.
Premenstrual-like syndrome.
2. *Breast.*
Tenderness, enlargement, secretion.
3. *Gastrointestinal.*
Cholestatic jaundice.
Vomiting, nausea.
Abdominal cramps, bloating.
4. *Skin.*
Hemorrhagic eruption.
Erythema nodosum.
Erythema multiforme.
Hirsutism.
Chloasma or melasma which may persist when drug is discontinued.
Loss of scalp hair.
5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.
6. *CNS.*
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. *Miscellaneous.*
Aggravation of porphyria.
Edema.
Reduced carbohydrate tolerance.
Increase or decrease in weight.
Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms —One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. *Given cyclically:*

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure —A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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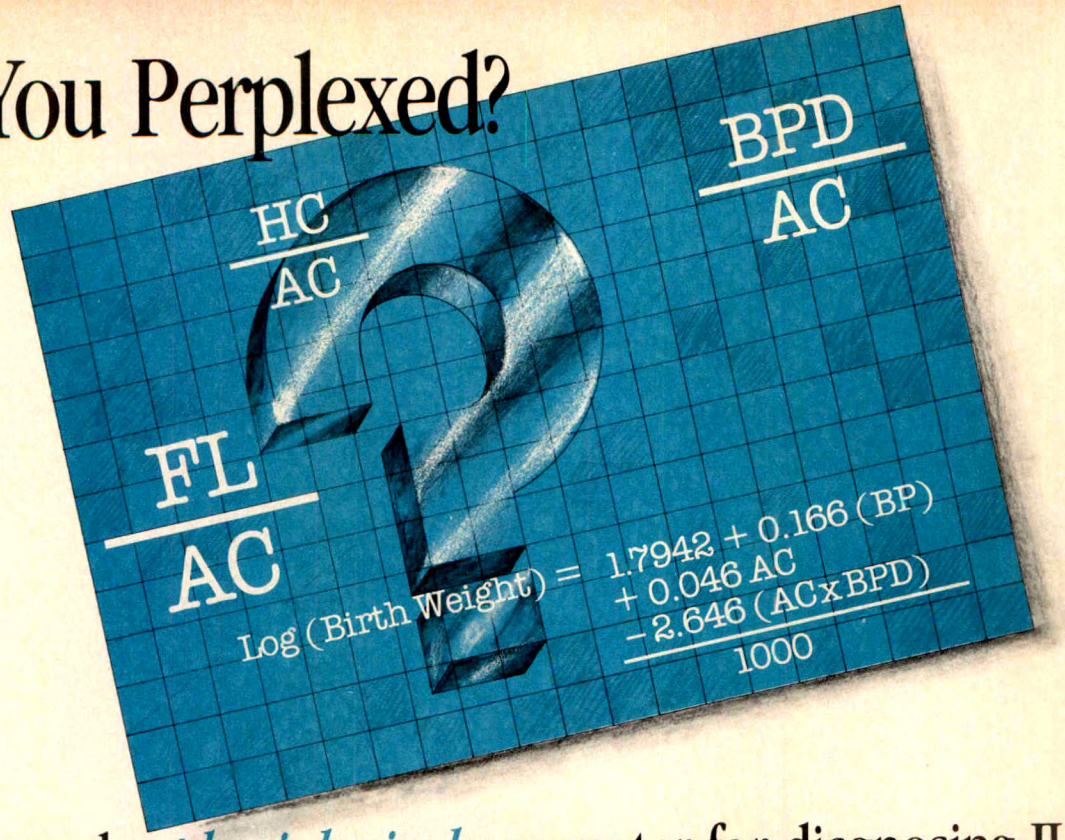
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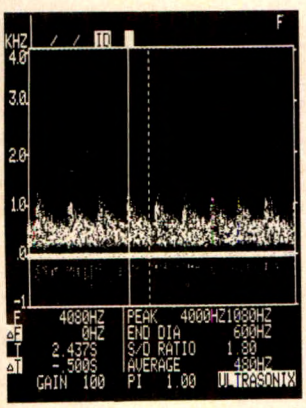
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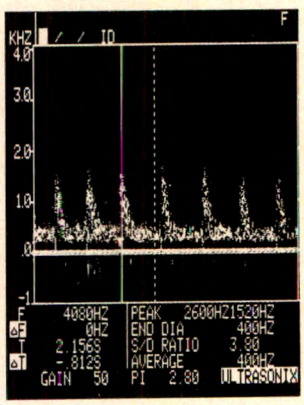
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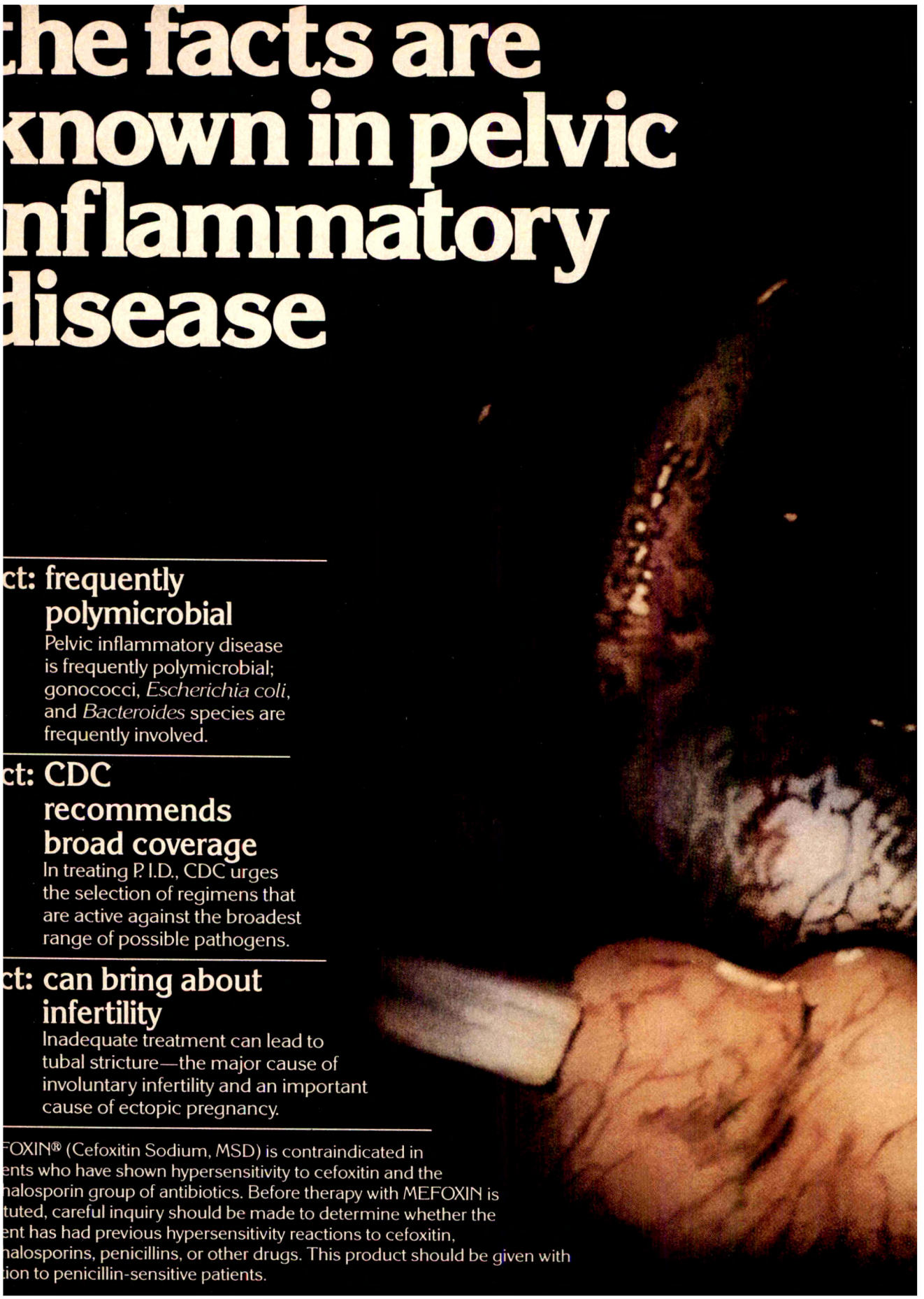
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†MEFOXIN is indicated for the treatment of pelvic inflammatory disease caused by *Escherichia coli*, *Neisseria gonorrhoeae* (penicillinase- and non-penicillinase-producing), *Bacteroides* species including the *Bacteroides fragilis* group, *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and Group B streptococci. MEFOXIN is not indicated for treatment of pelvic inflammatory disease where *Chlamydia trachomatis* is present or suspected.

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Indications and Usage: *Treatment*—Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:

LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia and lung abscess, caused by *Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), other streptococci (excluding enterococci, e.g., *Strep. faecalis*), *Staphylococcus aureus* (penicillinase and non-penicillinase producing), *Escherichia coli*, *Klebsiella* species, *Hemophilus influenzae*, and *Bacteroides* species.

GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST ‖ reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

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Ultrasound evaluation of polyhydramnios and twin pregnancy

Beverly Hashimoto, M.D., Peter W. Callen, M.D., Roy A. Filly, M.D., and Russell K. Laros, M.D.

San Francisco, California

We analyzed the ultrasound examinations and medical records of 75 pairs of twins who were delivered between January, 1983, and December, 1984, to study the relationship between increased amniotic fluid volume, fetal abnormalities, and preterm labor. Ten of these 75 twin pregnancies demonstrated elevated amniotic fluid volume that persisted throughout pregnancy. Total intrauterine volumes were elevated in these cases, and nine of the ten pregnancies were abnormal. In addition, it was noted that elevation of the amniotic fluid volume alone did not explain the high rate of preterm labor and delivery in twin gestations. (Am J Obstet Gynecol 1986;154:1069-72.)

Key words: Twins, polyhydramnios, abnormalities in twins, premature labor

Ultrasonographic identification of twin pregnancies is important because these pregnancies have much higher morbidity and mortality in comparison with singleton gestations.[1] Besides identification of twin gestations, ultrasound is also useful in assessing fetal well-being.[2] One well-known complication of twin pregnancy is increased amniotic fluid volume. Although polyhydramnios in singleton gestations has long been considered a possible ominous finding associated with multiple maternal and fetal complications,[3-6] there is a general perception that polyhydramnios in twins is a common, normal event.[7] We reviewed our 2-year experience to assess the frequency of polyhydramnios in our twin population and to determine whether the presence of polyhydramnios in twin pregnancies was associated with fetal abnormalities or could explain the increased incidence of preterm labor in twin gestations.

Material and methods

We reviewed the medical records and ultrasound examinations of all twin pregnancies in which either delivery or sonographic study took place at our institution from January, 1983, to December, 1984. A total of 86 twin pregnancies were identified. Eleven pregnancies were excluded for the following reasons: fetal death in the first trimester (two), and only one sonogram performed at our institution at less than 10 menstrual weeks (four), twins not suspected prenatally, so that no sonogram was performed (one), or medical records unavailable from the referring institution (four). Therefore 75 patients for whom both ultrasound examinations and delivery records were available were the basis for our study.

The medical records and ultrasound examinations were reviewed to ascertain presence of polyhydramnios, occurrence of preterm labor, congenital abnormalities, and referral source of the patient. The ultrasound examinations were the basis for determining the presence of polyhydramnios. We subjectively diagnosed "increased amniotic fluid volume" if the fetal body was completely separated from the anterior part of the uterine wall. Total intrauterine volumes were then calculated in all pregnancies in which there were adequate longitudinal and transverse static images obtained by a standard method previously described.[8] Total intrauterine volumes were obtainable in 55 of 75 pregnancies. Of 18 patients who were diagnosed subjectively as having increased amniotic fluid, total intrauterine volumes could be determined for 15 patients. The remaining three had gross polyhydramnios but could not be transported for static B scans. Our total intrauterine volumes were then compared with previously published total intrauterine volume data on twins.[9] Polyhydramnios was diagnosed only if the total intrauterine volume was elevated (above the normal standards set previously for twin gestations).[9]

Preterm labor was diagnosed if the medical record indicated significant contractions with ruptured membranes or cervical change before 37 menstrual weeks. All the women determined to have preterm labor were admitted and medically treated for variable periods. Since we were specifically interested in identifying those pregnancies that resulted in spontaneous preterm delivery, we excluded six patients in whom labor was induced: four because of severe fetal congenital anom-

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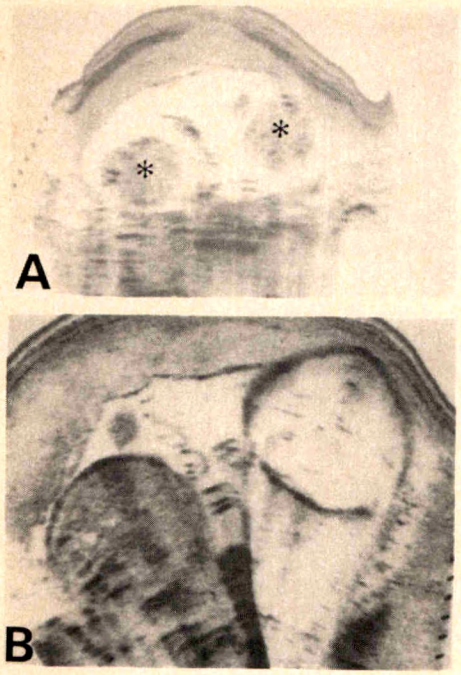


Fig. 1. Transiently increased amniotic fluid. *A*, At 22 weeks' gestation the amniotic fluid appeared increased (surrounding both fetal bodies *[asterisks]*). Total intrauterine volume was normal. *B*, The same patient demonstrates a normal amount of amniotic fluid at 32 weeks.

Table I. Abnormal pregnancies and polyhydramnios

Abnormality	*Polyhydramnios*	
	Yes	*No*
Acephalia and acardia	2	
Conjoined twins	2 pairs	
Bladder obstruction		1
Ambiguous genitalia		1
Diaphragmatic hernia		1
Abnormal hands		1
"Stuck" twin	5	

Table II. Relationship between polyhydramnios and preterm labor and delivery

	*Polyhydramnios**		
	Yes	*No*	*p Value*
Preterm labor			0.25
Yes	10	23	
No	8	34	
Preterm delivery			0.43
Yes	12	32	
No	6	25	

*Including patients with transient fluid elevation and constant polyhydramnios.

alies, one because of maternal preeclampsia, and one because of therapeutic abortion in the early second trimester.

Since our institution is a referral center, we reviewed medical records to determine the source and reason for referral in each case. The reasons for referral by outside physicians included suspicion of fetal abnormality by outside ultrasound examination, preterm labor or preterm rupture of membranes, and polyhydramnios.

Results

Increased amniotic fluid volume was diagnosed by visual inspection in 18 of 75 patients. Of these 18 patients, eight had transient increased amniotic fluid volume that resolved later in pregnancy (Fig. 1). The other 10 patients maintained elevated amniotic fluid volumes throughout pregnancy. We were able to calculate total intrauterine volumes for all eight patients with transient visual increase in amniotic fluid volumes and for seven of the ten patients with persistently elevated amniotic fluid volumes. The eight patients with transient increased amniotic fluid volume each had normal or upper limits of normal total intrauterine volumes. However, the seven patients with persistently elevated amniotic fluid volume all had abnormal total intrauterine volumes. The remaining three patients with persistently elevated amniotic fluid volumes were also placed in the polyhydramnios group, even though total intrauterine volumes could not be determined. Total intrauterine volumes were also calculated for 40 of the 57 patients who did not appear to have polyhydramnios. All of these patients had normal total intrauterine volumes.

Of the 75 pregnancies in our study, 13 were abnormal (Table I). In five pregnancies, one twin was in a polyhydramniotic sac and the other twin was in an oligohydramniotic sac.[10] The twin in the oligohydramniotic sac was usually growth retarded, and the other twin usually developed hydrops.[10] These cases are all presumed to be twin transfusion syndrome, although unambiguous pathologic or clinical confirmation could not always be obtained. Abnormal pregnancies other than cases of an oligohydramniotic sac in one twin involved fetuses with congenital anomalies. Congenital anomalies present in our series included acardiac, acephalic parabiotic twins (two fetuses), conjoined twins (two pairs of twins), diaphragmatic hernia (one fetus), bladder outlet obstruction (one fetus), abnormally formed hands (one fetus), and ambiguous genitalia (one fetus). Polyhydramnios was present in only one pregnancy with normal fetuses.

In the group with "transiently increased amniotic fluid volume," there were no abnormal fetuses. The lack of abnormal pregnancies and the persistently normal total intrauterine volumes indicate that poly-

hydramnios should not be considered in such pregnancies; rather, visual clues suggesting polyhydramnios in singleton pregnancies are too stringent to be applied to twins during certain phases of pregnancy development.

In our study, 44 of 75 women (59%) developed preterm labor, and 33 of these (44%) had preterm delivery. A χ^2 analysis of the relationship between preterm labor and delivery and patients with either polyhydramnios or transient amniotic fluid elevation showed no significant relationship between these factors (Table II).

Of the 75 patients in the study groups, 20 were referred from outside rather than through our own outpatient clinical practice. The reasons for referral included suspicion of fetal anomaly (nine patients), preterm labor or preterm rupture of the membranes (nine), and polyhydramnios (two). None of the eight patients with transient amniotic fluid elevation was referred, and only two of the ten patients with persistent polyhydramnios were referred specifically because of a suspicion of increased amniotic fluid. Among the nine patients referred because of a suspicion of a fetal anomaly, there were seven pregnancies with a documented anomaly, in one a "stuck twin" phenomenon was present, and one pregnancy was normal. Of these nine patients, five had polyhydramnios. Among 44 patients with preterm labor, nine were referred for either preterm labor or premature rupture of membranes. All of these referred patients experienced preterm delivery.

Comment

Polyhydramnios in singleton gestations has generally been defined as greater than 2 L of amniotic fluid within the gestational sac.[3, 5, 11] In the past, only invasive means, such as physical removal and measurement of the volume of fluid within the amniotic sac[12] or dilution measurements of amniotic fluid,[11] were available to diagnose polyhydramnios. Now with widespread availability of ultrasonography, this modality is generally used to diagnose polyhydramnios.[6]

Experienced ultrasonographers each have visual clues that alert them to the probability that a given pregnancy has an abnormally large amount of amniotic fluid. These visual clues vary throughout pregnancy because the proportional quantity of fluid in comparison with fetal size alters with progress toward term. We, as others, have successfully used such clues but have learned during the course of this study that impressions largely derived from observation of singleton pregnancies cannot be transposed to twin gestations. Obviously our subjective criteria are unique to our group. Had other criteria been utilized, such as the "8 cm rule," the results might have met with varying degrees of success. Likewise, although there was no attempt to subjectively grade the degrees of polyhydramnios, in those groups in which polyhydramnios would have been interpreted as severe, there would undoubtedly have been a correlation with an abnormally increased total intrauterine volume.

Using ultrasound for evaluating increased amniotic fluid volumes, we have identified two groups of twin pregnancies. One group had a high frequency of abnormal pregnancies. All the subjects in this group had strong visual clues suggesting polyhydramnios and abnormally high total intrauterine volumes throughout pregnancy. The other group subjects, with normal pregnancies, had visual clues suggesting elevated amniotic fluid volume in midtrimester that disappeared later in pregnancy. Moreover, total intrauterine volumes were normal in all these patients throughout pregnancy. Although the utility of measuring total intrauterine volume has been limited in some studies, this measurement appears to be useful in discriminating between those twin pregnancies in which amniotic fluid volume is normal and those in which polyhydramnios is present. It is of interest that other studies utilizing subjective methods to diagnose polyhydramnios also note similar dichotomies in their polyhydramnios groups.[6] It is recognized, however, that for many examiners the unavailability of static articulated arm equipment may not make this measurement practical.

It now seems clear that visual clues employed to diagnose polyhydramnios in singleton gestation, such as the ones used in this study, are not applicable to twin gestations. Amniotic fluid may normally occupy two thirds of the gestational sac in a singleton gestation at 20 weeks.[3] Two sacs with fluid at the upper limits of normal confined within the uterus would likely simulate polyhydramnios. Likewise it is conceivable that in those cases in which there was polyhydramnios in one sac and oligohydramnios in the other, the abnormal sac volumes might tend to neutralize one another and the total intrauterine volume might be normal. Moreover, in one of the eight cases with "transient amniotic fluid elevation," only one sac appeared to have excess fluid. This observation further supports our contention that the subjective determination of polyhydramnios in twin pregnancy is too broad. Since the calculation of total intrauterine volumes appeared to differentiate between these groups, this calculation may be useful in diagnosing polyhydramnios more strictly than a subjective method.

If one considers only the group with abnormally elevated total intrauterine volumes as having polyhydramnios, the frequency of abnormally increased fluid volume is still substantially higher than in singleton gestations (10 of 75 cases).[3, 5] Since our institution is a referral center, there was potential for referred cases to dramatically influence the prevalence of polyhydramnios in our patient population—and they did so.

Eight of twenty referred patients carrying twins had polyhydramnios, whereas only two of 55 nonreferred patients had polyhydramnios. The prevalence in our nonreferred population was only 3.6%. Previous studies have found that polyhydramnios is present in 6% to 7% of twin pregnancies.[13] When one considers that two pregnancy events occur in twinning, one would expect the prevalence of polyhydramnios to be twice that in singleton pregnancies. This is approximately correct in our nonreferred patient group.

In our patient group with clearly defined polyhydramnios (i.e., strong visual clues, persistently elevated fluid quantities, and abnormal total intrauterine volume), nine of 10 cases were abnormal. Other series have noted that 30% to 70% of twin pregnancies in which polyhydramnios is present are abnormal.[3]

Increased uterine distension has been considered one possible cause for preterm labor and delivery,[14] and this mechanism has been used to explain the increased frequency of preterm labor and delivery in multiple gestations.[15] Since polyhydramnios would cause increased distension and is thought to be common in twin pregnancies, we attempted to determine whether there is a relationship between preterm labor and delivery and polyhydramnios. The frequency of preterm delivery in our study (44%) is within the broad range of 30% to 86% found in other studies.[16] However, no statistical relationship was found between preterm delivery and polyhydramnios in our series.

In summary, polyhydramnios is not a common finding in "normal" twin pregnancies. If polyhydramnios is diagnosed, the pregnancy should be closely studied to exclude abnormalities. Although diagnosis of polyhydramnios is important, subjective sonographic estimation of fluid in twin pregnancies was difficult in our hands. We found that determination of total intrauterine volumes was more accurate in segregating twin pregnancies in which polyhydramnios is present from those in which fluid volume is at the upper limits of normal.

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The role of lymphangiography in vulvar carcinoma

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The records of all patients with vulvar carcinoma seen at Washington University School of Medicine between 1970 and 1983 were reviewed to determine the role of lymphangiography in the management of patients with vulvar malignancy. Forty-three patients were identified who had a preoperative lymphangiogram followed by radical vulvectomy and lymph node dissection. Thirty-two films were available for review. Seventy-seven sets of lymph nodes were obtained from the 32 patients. Overall diagnostic accuracy was 42 of 77 (54.5%), with a sensitivity of three of 19 (15.7%) and a specificity of 39 of 59 (66.1%). Corresponding values for inguinal nodes were 26 of 57 (45.6%), two of 16 (12.5%), and 24 of 41 (58.5%), respectively. Accuracy rates for pelvic nodes were 16 of 21 (76.2%) with a sensitivity rate of one of three (33%) and a specificity of 15 of 18 (83%). Negative scans were more likely to be accurate than positive scans, 88.6% versus 30%. While a negative lymphangiogram may be helpful in predicting the absence of metastases, its poor specificity limits its widespread usefulness. (Am J Obstet Gynecol 1986;154:1073-5.)

Key words: Vulvar carcinoma, lymphangiography

The accepted management of invasive carcinoma of the vulva has traditionally been radical vulvectomy and en bloc resection of the regional lymphatics and inguinal lymph nodes. Recently, investigators have been attempting to define a subset of patients who might benefit from a less radical surgical approach.[1] An important adjunct to such individualized treatment planning would be the ability to detect those patients likely to have lymph node metastases. The use of lymphangiography in the preoperative workup of these patients has been controversial, with accuracy ranging from 60% to 85%.[2-7]

Bipedal lymphangiography has been part of the routine preoperative evaluation of patients scheduled to undergo radical vulvectomy and lymph node dissection at Barnes Hospital, St. Louis, Missouri. This experience has been reviewed to determine the potential role of lymphangiography in the treatment planning of patients with vulvar malignancy.

Material and methods

Patients. The records of all patients with vulvar carcinoma between the years 1970 and 1983 were reviewed. Of 58 patients, 43 were identified who had a preoperative lymphangiogram followed by a radical surgical procedure and a lymphadenectomy. Thirty-two of these patients had films available for review by one of the authors (J.K.T.L.); the radiographs on the other 11 patients were unobtainable and therefore not included in the final statistical analysis.

Technique of lymphangiography. Bipedal lymphangiography was performed in a standard fashion as reported previously.[4] Briefly, 0.5 ml of alphazurine A was injected intradermally into the web space between the first and second toes and between the third and fourth toes to facilitate the identification of lymphatic vessels. A 1-inch-long incision was made over the dorsum of the foot; a lymphatic vessel was isolated and cannulated with a 27- or 30-gauge needle. Ethiodol, 5 to 7 ml, was injected into each lymphatic vessel with an infusion pump.

Radiographs of the pelvis and abdomen in both frontal and oblique projections were made immediately after injection as well as 24 hours after the procedure.

Operative technique. Twenty-eight patients underwent the standard Basset operation—radical vulvectomy with en bloc removal of superficial and deep inguinal lymph nodes. Within this group, eight had bilateral retroperitoneal pelvic lymphadenectomies to the level of the bifurcation of the common iliac arteries. Three patients had exenterative procedures (two posterior, one anterior) that included transperitoneal pelvic lymphadenectomies. One patient had a bilateral inguinal lymphadenectomy only, having previously undergone a vulvectomy. In each patient, lymph nodes were separated both radiologically and pathologically into four groups, right and left inguinal and right and left pelvic. Diagnostic accuracy of lymphangiography was assessed by comparing radiologic findings with his-

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Table I. Combined lymphangiography

Histologic diagnosis	Radiologic diagnosis			Total
	Positive	Equivocal	Negative	
Positive	3	11	5	19
Negative	7	13	39	59
Total	10	24	44	

Table II. Inguinal lymphangiography

Histologic diagnosis	Radiologic diagnosis			Total
	Positive	Equivocal	Negative	
Positive	2	10	4	16
Negative	6	11	24	41
Total	8	21	28	57

tologic findings for each of the four groups of lymph nodes.

Radiographic criteria. The following criteria were used when the lymphangiograms were interpreted: (1) A lymph node was considered to contain metastases if a nodal defect was peripheral in location, if it was 0.5 cm in diameter, or if it was not traversed by lymphatic channels. (2) A lymph node was considered to be indeterminate for metastases if it was large (>2 cm) but had no focal defect, if it had a central filling defect, or if it had a peripheral defect <3 mm in size. (3) A lymph node was considered to be negative if it had had none of these findings.

Statistics. The following definitions were used: (1) Sensitivity equals the number of positive examinations in cases with metastases divided by the number of cases with metastases. (2) Specificity equals the number of negative examinations in cases without metastases divided by the number of cases without metastases. (3) True positive examinations equal the number of cases with metastases and positive examinations divided by the number of positive examinations. (4) True negative examinations equal the number of cases without metastases and negative examinations divided by the number of negative examinations. The 95% confidence intervals were calculated where indicated.

Results

Lymphangiography correctly predicted pathologic findings in 42 of 77 cases, for an accuracy rate of 53.8%. The overall sensitivity (positive examinations in patients with metastases) was three of 19 (15.7%, with a 95% confidence interval of 4% to 39%), whereas the specificity (negative examinations in patients without disease) was 39 of 59 (66.1%, 95% confidence interval 52% to 78%) (Table I). The likelihood that a negative scan correlated with absence of metastases was 39 of 44 (88.6%, 95% confidence interval 74% to 97%), while a positive scan correctly predicted metastases in three of 10 (30%, 95% confidence interval 7% to 65%) instances.

Overall accuracy for inguinal nodes was 26 of 57 (45.6%); two of 16 (12.5%, 95% confidence interval 2% to 36%) patients had nodal metastases detected whereas 24 of 41 (58.5%, 95% confidence interval 42% to 75%) patients without disease were correctly identified (Table II). The predictability of a negative inguinal examination was 24 of 28 (85.7%, 95% confidence interval 68% to 96%), whereas a positive scan was correct in only two of eight cases (25%, 95% confidence interval 3% to 65%).

Table III. Pelvic lymphangiography

Histologic diagnosis	Radiologic diagnosis			Total
	Positive	Equivocal	Negative	
Positive	1	1	1	3
Negative	1	2	15	18
Total	2	3	16	21

The specificity and sensitivity of lymphangiography in detecting patients with pelvic node metastases, although not statistically significant, were somewhat better than in patients with inguinal node disease. The accuracy rate for pelvic nodes alone was 16 of 21 (76.2%). The specificity was 15 of 18 (83%, 95% confidence interval 58% to 96%), while the sensitivity was one of three (33%, 95% confidence interval 0% to 76%) (Table III). The predictability of a negative scan was similar to that of inguinal lymphangiography, 15 of 16 (93.75%, 95% confidence interval 70% to 100%), whereas correlation of a positive scan with the presence of metastases was difficult to determine because of small numbers (one of two or 50%, 95% confidence interval 0% to 100%).

Equivocal examinations accounted for 36.8% of all inguinal lymphangiograms but for only 14.2% of pelvic scans. Of the equivocal examinations, 54.1% were without documented metastases and 45.8% had histologic evidence of disease.

Comment

Two areas of controversy exist in the treatment of patients with invasive vulvar carcinoma. The first involves identifying those patients with "microinvasive" and "early" carcinoma who are at low risk for inguinal metastases and could be treated with a limited excision of the primary lesion.[1] The second area relates to identifying those patients with pelvic node metastases who would require alteration of regional therapy to include treatment of pelvic lymph nodes.

Lymphangiography in the evaluation of the gynecologic oncology patient has been in use since 1958,[2]

and early reports of its accuracy were quite favorable.[3,4] Subsequently, in 1965, Averette[5] reported that 61 of 82 patients (73.4%) with gynecologic cancer had accurate lymphangiographic diagnosis of nodal metastases. In 1969, Comas et al.,[6] from the same institution, reported an accuracy rate of 85% in 20 patients with vulvar carcinoma. In 1970, Hagen and Bjorn-Hansen[7] reported on 29 patients with vulvar carcinoma and found an accuracy rate of 60.5% in detecting inguinal node metastases, with 12.5% false positive and 12.5% false negative results and an equivocal rate of 14.5%.

In the current series, the sensitivity of lymphangiography in detecting lymph node metastases, either pelvic or inguinal, was extremely poor. A test that can only detect metastatic disease less than 33% of the time can hardly be considered useful in identifying patients with nodal metastases. Even in the presence of a positive scan, only 25% to 50% of patients were found to have evidence of metastatic nodal disease.

Although lymphangiography appears unable to accurately identify patients with metastases, clinical usefulness could still be claimed if patients without metastatic disease could be detected. In this regard, the examination is more accurate. Although not statistically significant, pelvic lymphangiography, with a specificity of 83%, appears to be somewhat more useful than inguinal lymphangiography, with a specificity of 58.5%. Even more important, a negative pelvic or inguinal scan has an accuracy rate of 85% to 90%. The major point of departure between pelvic and inguinal examinations appears to be that inguinal lymphangiography has a large number of equivocal examinations (36.8%), making it less useful as a diagnostic procedure. Our findings seem to be in agreement with those of a number of investigators[6-8]; interpretation of metastases to inguinal nodes is fraught with difficulty. The presence of inflammatory changes, fibrolipomatosis, incomplete filling of nodal chains, and anatomic variability all make the task of radiologic interpretation of inguinal nodes extremely difficult.

In summary, the role of lymphangiography in vulvar carcinoma is extremely limited. Its inability to detect inguinal node metastases precludes its use in identifying patients who need an inguinal node dissection. Although negative examinations are fairly accurate (85.7%), the low specificity and large number of equivocal examinations in the inguinal region would result in a large number of patients without metastases being managed as if they had metastatic disease. In regard to pelvic lymphangiography, the small numbers of patients with positive examinations and metastatic nodes make conclusions difficult, but the trends tend to indicate an inability to predict metastases. However, because the specificity of pelvic lymphangiography is somewhat higher and only 14% of examinations were equivocal, pelvic scans may have a role in identifying patients with vulvar carcinoma who are unlikely to have pelvic metastases, as a negative examination had an accuracy rate of 93.7%.

Assuming one believes that pelvic lymphadenectomy is indicated in the presence of inguinal metastases, one could use a negative pelvic lymphangiogram to exclude patients from this extended surgical procedure. Since only 20% to 25% of patients with positive inguinal nodes will have pelvic metastases,[9] a negative examination would give approximately 90% assurance that there is no metastatic disease. One could therefore avoid the more radical operation, which is associated with increased morbidity and questionable survival benefits.[9,10] If pelvic lymphadenectomy is not contemplated in the primary therapy of vulvar carcinoma, then it would appear that lymphangiography has very little role to play in the management of the patient with this disease.

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Human fallopian tube contains placental protein 14

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Radioimmunoassay and immunoperoxidase staining were used to study the tissue content and localization of an endometrial protein, placental protein 14, in the human fallopian tube. Placental protein 14 immunoreactivity was found in saline extracts from all fallopian tubes tested ($n = 14$). In the fimbrial part the placental protein 14 content was higher in the secretory than in the proliferative phase ($p < 0.01$). No difference was found in the placental protein 14 content between the isthmic, ampullar, and fimbrial parts of the tube. Immunoperoxidase staining localized placental protein 14 to the ciliated and secretory epithelial cells of the mucosa in all parts of the tube regardless of the phase of menstrual cycle. The occurrence of the same protein in the endometrium and fallopian tube is compatible with the common embryonic origin from the müllerian duct of these tissues. (Am J Obstet Gynecol 1986;154:1076-9.)

Key words: Endometrial proteins, placental protein 14, human fallopian tube

Placental protein 14 belongs to a group of proteins isolated from the placenta and its membranes.[1] Placental protein 14 was recently detected in the serum of nonpregnant women, where the levels show consistent variation during the menstrual cycle.[2] Since placental protein 14 was recently found to be endometrial in origin[3, 4] and since the fallopian tubes and the uterus derive from the same embryonic source, we studied human fallopian tubes for their placental protein 14 content in various phases of the menstrual cycle.

Material and methods

Samples. Salpingectomy and hysterectomy were performed on 14 apparently healthy nonpregnant women because of uterine fibroids. None of the women received hormone treatment. After the tissues had been removed and representative specimens taken for routine histopathologic examination, additional specimens from the fallopian tubes were separated for this study. In nine cases, representative samples of the ampullar, isthmic, and fimbrial parts of the tube were fixed in formalin for immunoperoxidase staining, and in all 14 cases, specimens were obtained from the three parts for the analysis of tissue placental protein 14 content by radioimmunoassay. These tissues were immediately frozen in liquid nitrogen and stored there until tested. In 10 cases a serum sample was obtained before operation and stored at −20° C until studied. The tissues were weighed, homogenized in phosphate-buffered saline solution (pH 7.4 at 4° C) containing 175 mg/L of phenylmethylsulfonylfluoride (Merck, Darmstadt, West Germany) as a protease inhibitor, and centrifuged at 4° C (10,000 × g, 30 min). The supernatants were used for protein and placental protein 14 determinations.

Radioimmunoassays. The radioimmunoassay for placental protein 14 has been described previously.[4] Purified placental protein 14 (lot No. 120/135, Behringwerke AG, Marburg, West Germany) was iodinated by the chloramine-T method.[5] Placental protein 14 antiserum (lot No. 201ZA, Behringwerke) was used at a dilution of 1:9000. The detection limit of the assay was 8 μg/L. Apart from 1% crossreactivity with purified placental protein 12, the anti–placental protein 14 antiserum did not crossreact with any known placental or human serum protein.[1, 4] The total protein content of the tissue extracts was estimated by the Lowry method.[6] Serum progesterone was estimated by a commercial radioimmunoassay kit (Farmos Diagnostica, Oulunsalo, Finland).

Gel filtration. To find out whether placental protein 14 forms complexes or is degradated in fallopian tubal extract, 125 iodine–placental protein 14 was incubated with the extract at 37° C for 4 hours. Chromatography was carried out on a Sephadex-G100 column (60 × 1 cm, Pharmacia, Uppsala, Sweden), and the radioactivity of the fractions was counted. The molecular size of fallopian tubal placental protein 14 was estimated by gel filtration on the same column and compared with that of ^{125}I-labeled placental protein 14. The column

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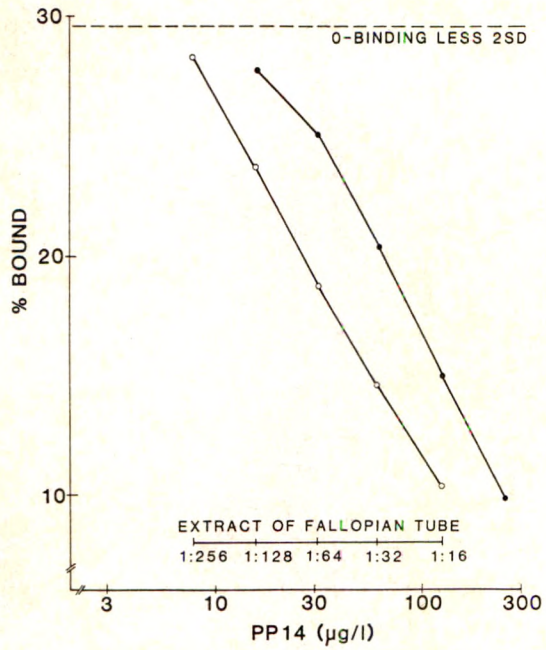


Fig. 1. Dose-response curves of purified placental protein 14 *(PP14)* (●——●) and fallopian tubal extract (○——○).

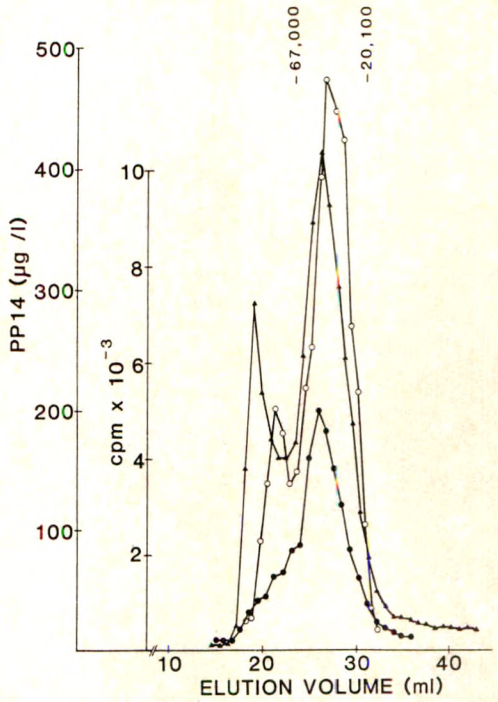


Fig. 2. Gel filtration of fallopian tubal extract (○——○), purified ^{125}I-labeled placental protein 14 (●——●), and ^{125}I-placental protein 14 incubated with the extract of fallopian tube (▲——▲). Molecular weight markers: bovine serum albumin (molecular weight, 67,000) and soybean trypsin inhibitor (molecular weight, 20,100).

was calibrated with bovine serum albumin (Sigma, St. Louis) and soybean trypsin inhibitor (Boehringer, Mannheim, West Germany).

Histopathologic examination and immunoperoxidase staining. Histologic examination of the endometrial specimens was performed in order to accurately date the phase of the cycle. The immunoperoxidase staining procedure has been described elsewhere.[7]

Statistical analyses were performed by the Wilcoxon rank sum test and the Spearman rank correlation test.

Results

Placental protein 14 content in the fallopian tubes. Placental protein 14–like immunoreactivity was detected in all parts of all the fallopian tubes examined (Table I). In the fimbrial part the values were higher in the secretory than in the proliferative phase ($p < 0.01$). No difference was observed in the placental protein 14 content of different parts of the tube, and no correlation was found between the serum placental protein 14 and progesterone concentrations or between the tissue content of placental protein 14 and the serum progesterone concentration.

In radioimmunoassay, serial dilutions of purified placental protein 14 and fallopian tubal extract gave parallel dose-response curves (Fig. 1). In gel filtration, tubal placental protein 14 eluted as two peaks. The first peak represents possibly an aggregated form, whereas the second peak corresponds to the elution volume of purified ^{125}I-labeled placental protein 14. Incubation of ^{125}I-labeled placental protein 14 with tubal extract yielded a similar elution pattern (Fig. 2).

Immunohistochemical localization. The endometrium was proliferative in seven cases and secretory in another seven. The fallopian tubes did not exhibit similar morphologic changes. Irrespective of this, all the fallopian tubes contained placental protein 14 demonstrable by the immunoperoxidase method. Placental protein 14 was localized in the ciliated and secretory epithelial cells of the mucosa (Fig. 3). Staining was similar in all parts of the fallopian tube regardless of the phase of menstrual cycle.

Comment

We report here results by immunochemical and immunohistochemical methods on the occurrence of placental protein 14–immunoreactive material in the human fallopian tube. The tubal placental protein 14 content was higher in the secretory than in the proliferative phase. This is compatible with our recent observations on the endometrium.[3]

The fallopian tubes and the uterus both derive from the müllerian duct. Therefore it was not surprising to find placental protein 14 in both of these tissues. Our recent studies indicate that human secretory endometrium is capable of synthesizing placental protein 14.[3]

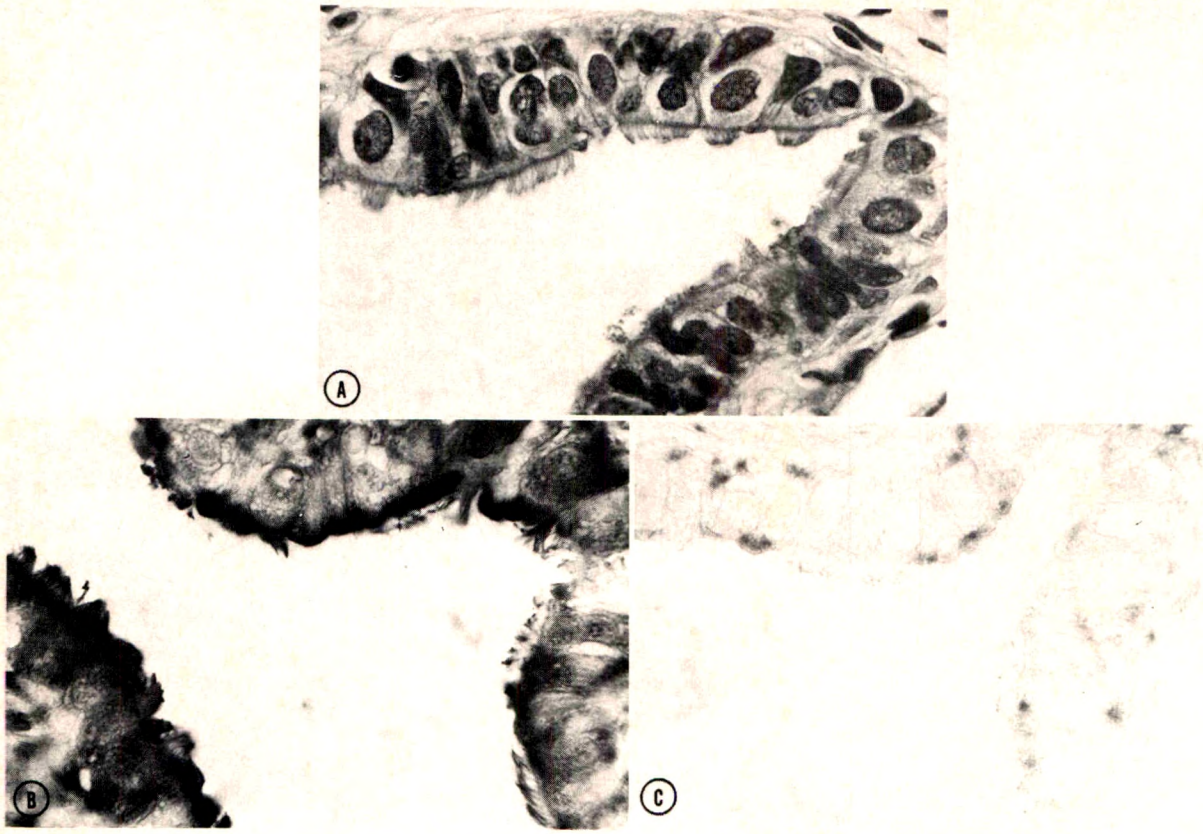


Fig. 3. Immunoperoxidase staining of fallopian tube, ampullar part. *A* shows a hematoxylin and eosin stain. In *B* the adjacent section has been stained by immunoperoxidase for placental protein 14. The positive reaction is seen both in the ciliated and the secretory epithelial cells of the mucosa. In *C* the next adjacent section has been stained with anti–placental protein 14 antiserum absorbed with purified placental protein 14 (50 μg/ml antiserum diluted 1 : 100) which blocks the stain reaction (magnification ×400).

Table I. Placental protein 14 concentration in tubal extracts and serum and the corresponding serum progesterone levels

Endometrial tissue	*Fallopian tubal placental protein 14 (μg/gm of protein)*			*Serum placental protein 14 (μg/L)*	*Serum progesterone (nmol/L)*
	Isthmus	*Ampulla*	*Fimbriae*		
Proliferative	2.3	3.3	1.5	<16	<1
Proliferative	5.2	17.3	6.5	<16	<1
Proliferative	1.6	4.0	3.8	42	<1
Proliferative	6.2	5.3	3.6	39	1.0
Proliferative	3.4	3.3	7.1	42	<1
Proliferative	19.8	25.9	ND	76	<1
Proliferative	ND	4.3	2.3	ND	ND
Median	4.3	4.3	3.7*		
Secretory	15.6	77.6	4.6	39	<1
Secretory	2.6	1.9	7.2	29	20
Secretory	7.3	12.6	33.1	ND	ND
Secretory	20.9	28.5	18.6	63	<1
Secretory	7.4	13.9	16.1	33	<1
Secretory	55.9	123.7	30.6	ND	ND
Secretory	9.6	16.8	13.0	ND	ND
Median	9.6	16.8	16.1*		

ND = Not done.
*$p < 0.01$ between proliferative and secretory phases.

Unlike the endometrium, the fallopian tube does not exhibit morphologic cyclic changes, and the variation of the placental protein 14 content was far less pronounced in the tube than in the endometrium. Recent comparison between placental protein 14 and progestagen-dependent endometrial protein[8] has shown that these two proteins are immunologically indistinguishable.[9] This adds interest to the occurrence of the protein in tubal mucosa. Placental protein 14 has also been identified in the fluids surrounding both the ovum and the sperm.[10, 11] It would be important to learn whether placental protein 14 in fallopian tube has anything to do with the transport of sperm or fertilized ovum.

We wish to thank Dr. Hans Bohn, Marburg, West Germany, for purified placental protein 14 and the antiserum. Technical assistance by Ms. Riitta Koistinen, M.Sc., Ms. Lea Armassalo, and Ms. Tuula Halmesvaara is gratefully acknowledged. This study was approved by the Joint Ethical Committee of Departments I and II of Obstetrics and Gynecology, Helsinki University Central Hospital.

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The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells

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It has been suggested that fetal adrenal steroids affect the secretion of progesterone by the human trophoblast and decrease the progesterone/estrogen secretory ratio at the time of parturition. In the present study, cultured human choriocarcinoma JEG-3 cells were used as an experimental model in order to examine the effect of dehydroepiandrosterone sulfate on both de novo and low-density lipoprotein–stimulated progesterone secretion. In serum-free and cholesterol-free Dulbecco's modified Eagle's medium, JEG-3 cell cultures demonstrated a significant secretion of both pregnenolone and progesterone. A 200% to 300% increase in pregnenolone and progesterone secretion was achieved by physiologic low-density lipoprotein concentrations added to Dulbecco's modified Eagle's medium while androgens and estrogens remained undetectable. Dehydroepiandrosterone sulfate added to Dulbecco's modified Eagle's medium was actively converted into C-19 and C-18 steroids but had no significant effect (up to 20 μg/ml) on basal (de novo) progesterone secretion. In contrast, the addition of dehydroepiandrosterone sulfate (1 to 5 μg/ml) to cultures grown in Dulbecco's modified Eagle's medium plus low-density lipoprotein induced a dose-related inhibition of progesterone and a return of that secretion to basal levels. (Am J Obstet Gynecol 1986;154:1080-5.)

Key words: Dehydroepiandrosterone sulfate, low-density lipoprotein, choriocarcinoma, progesterone

The mechanisms whereby the fetus of many species signals its readiness to enter extrauterine life involve an alteration in the fetal adrenal-trophoblast relationship. In the sheep, for example, a rise of cortisol induces changes in estrogen and progesterone secretions that are thought to be critical in increasing uterine prostaglandin activity.[1] In the human, because of the lack of 17-hydroxylase and 17,20-lyase activities in the trophoblast, the production of estrogens depends on adrenal steroids.[2,3] The possibility that dehydroepiandrosterone sulfate (DHEA-S) also influences the production of progesterone and alters the progesterone/estrogen secretory ratio at the time of parturition has been suggested.[4]

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Cultured human choriocarcinoma JEG-3 cells retain several secretory characteristics of the normal trophoblast. For example, they secrete human chorionic gonadotropin (hCG), free α-subunit of hCG, and progesterone and form estrogens when a C-19 steroid precursor is added to their culture medium.[5,6] Choriocarcinoma cells also display several of the cell surface receptors observed on the normal tissue and have receptors for low-density lipoprotein (LDL), a carrier of cholesteryl esters, which are important precursors of progesterone.[7,8] In the present study, JEG-3 cells were used as an experimental model in order to examine the influence of DHEA-S on progesterone secretion. This was studied under two conditions: (1) de novo progesterone formation, when cells are grown in serum-free and cholesterol-free synthetic medium, and (2) production of progesterone dependent on preformed cholesterol after addition of LDL to the culture medium.

Material and methods

JEG-3 cells, provided by Dr. Peter Kohler (Department of Medicine, University of Arkansas, Little Rock, Arkansas), were grown in 25 cm^2 culture plastic flasks in Dulbecco's modified Eagle's medium (DMEM) enriched with 10% heat-inactivated fetal calf serum at 37° C, in a 90% air–10% carbon dioxide atmosphere.

Fetal calf serum and DMEM were purchased from Gibco Laboratories, Grand Island, New York. Before each experiment, cells were collected by scraping, seeded to replicate flasks, and fed with DMEM supplemented with 10% fetal calf serum. At 30% to 50% confluency, media were changed to fetal calf serum–free DMEM. After 2 days in that medium, cultures were refed with DMEM alone or DMEM plus various experimental agents. Unless otherwise stated, culture media were collected 24 hours later, and after addition of bovine serum albumin (2 mg/ml) were stored frozen until measured for their steroid content. The following steroids were measured by specific radioimmunoassays after extraction from culture media with ethyl ether or petroleum ether and isolation by chromatography on Sephadex LH-20 (Pharmacia, Piscataway, New Jersey) columns: progesterone, 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione, testosterone, estrone, and 17β-estradiol. In each individual radioimmunoassay, recovery was evaluated by the addition of a trace radioactive quantity of the steroid to be measured to culture medium samples, and results were calculated accordingly. The procedure used for the above radioimmunoassays (routinely used in our clinical laboratory for the measurement of steroid levels in blood samples) has been reported.[9] The measurement of pregnenolone was performed by Dr. John Holt (Department of Obstetrics and Gynecology, University of Chicago) by means of a radioimmunoassay kit purchased from Radio Systems Laboratories, Carson, California. Samples were extracted with hexane, and pregnenolone was isolated by means of Celite microcolumns. The radioimmunoassay was performed with a rabbit antiserum raised against pregnenolone-3-monohemisuccinate coupled to human serum albumin. The cross-reactivity stated for progesterone is 1.5%, and cross-reactivities for dehydroepiandrosterone, androstenedione, testosterone, estrone, and estradiol are all below 0.01%. The cell sheets remaining in the culture flasks were washed with 0.9% sodium chloride and solubilized in 0.5N sodium hydroxide; an aliquot of each cell digest was used for protein determination.[10] Each determination represents the mean ± SEM of steroid secreted per milligram of cellular protein per 24 hours in at least three flasks, and the results shown are representative of at least three separate experiments. The two-sample *t* test was used for statistical analysis.

LDL was isolated from human male plasma according to the procedure of Goldstein and Brown.[11] Fresh LDL was prepared about every 2 weeks and stored at 4° C in a nitrogen atmosphere. The protein and cholesterol content of the LDL preparations was measured by the methods of Meulemans[12] and Allain et al.,[13] respectively. LDL was radioiodinated according to the

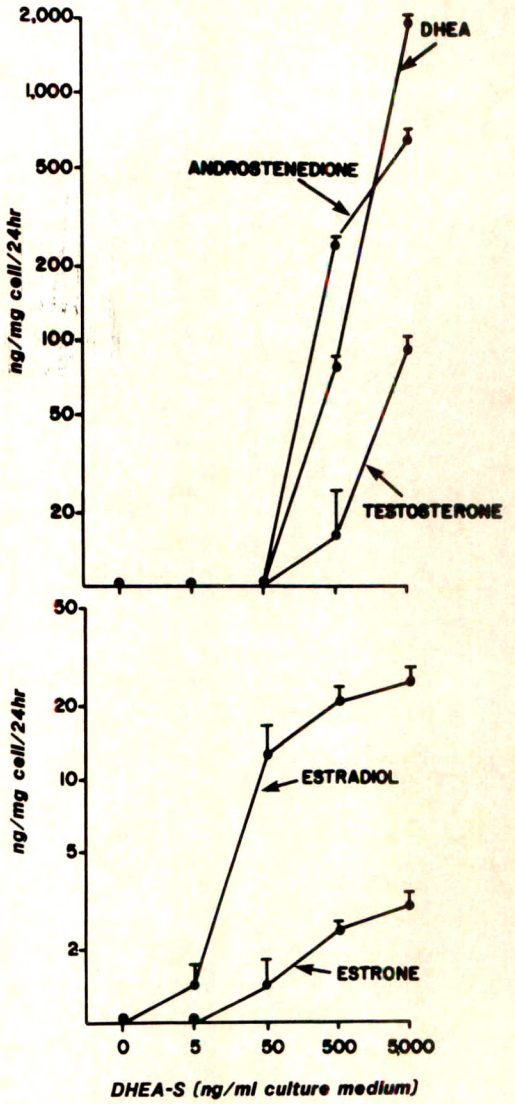


Fig. 1. Effect of DHEA-S on the secretion of C-19 and C-18 steroids by JEG-3 cells. After 3 days in serum-free DMEM, culture media were replaced by DMEM alone or DMEM plus DHEA-S. After 24 hours, media were collected for the measurement of dehydroepiandrosterone *(DHEA)*, androstenedione, and testosterone *(upper panel)* or for the measurement of estrone and estradiol *(lower panel)*, and the cell sheets were collected for protein measurement. Each point represents the mean ± SEM of three culture flasks.

procedure reported by Bilheimer et al.[14] The separation of unreacted iodine 125–labeled sodium from radiolabeled protein was achieved by means of a 24-hour dialysis (with a membrane of 3500 dalton nominal molecular weight cutoff) against 2 × 3 L of a solution containing 0.2 mol/L sodium chloride, 0.02 mol/L potassium iodide, and 0.001 mol/L ethylenediaminetetraacetic acid in water, pH 7.4, and a final dialysis against 1 L of DMEM. A specific activity of about 600 cpm/ng of protein was achieved. Uptake and degradation of [^{125}I]-LDL were evaluated at 37° C during a 24-hour

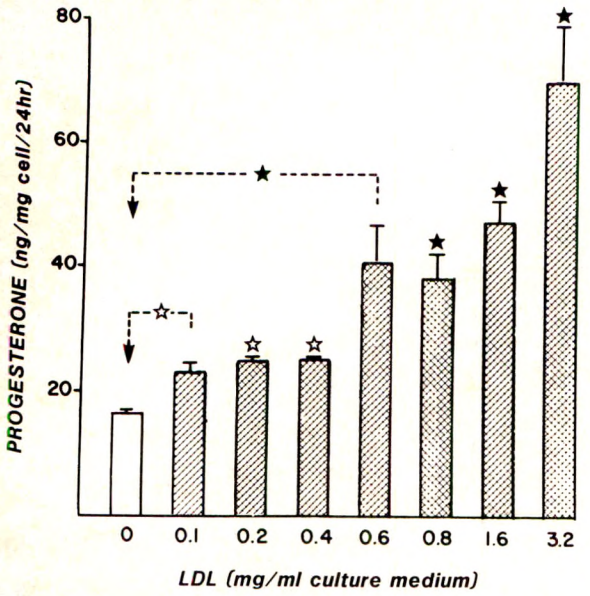


Fig. 2. Effect of LDL on progesterone secretion. After 3 days in DMEM, culture media were replaced by fresh DMEM *(open bar)* or DMEM plus LDL at various concentrations *(hatched bars)*. After 24 hours, media were collected for the measurement of progesterone (nanograms per milligram of cellular protein per 24 hours, mean ± SEM). *Open star* = $p < 0.025$; *solid star* = $p < 0.005$.

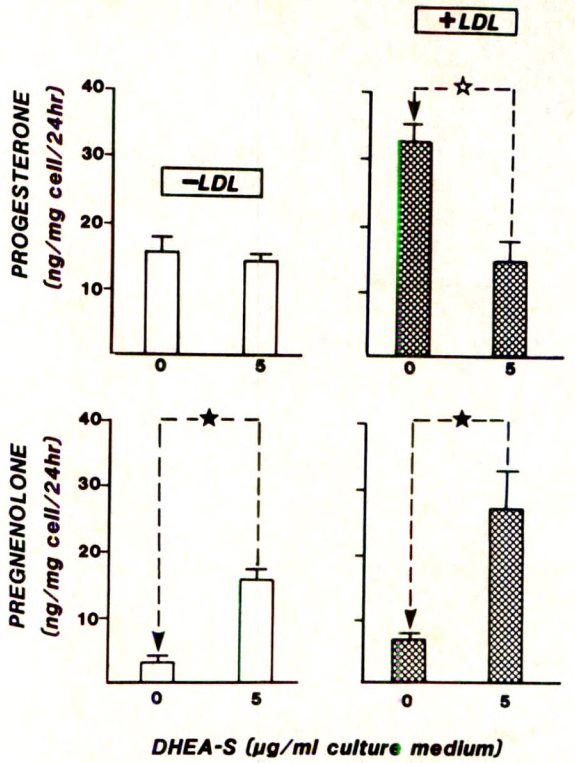


Fig. 3. Effect of DHEA-S on the secretion of progesterone *(upper panels)* and pregnenolone *(lower panels)* by cell cultures grown either in DMEM *(open bars)* or in DMEM plus LDL *(hatched bars)*. Remainder as in legend to Fig. 2.

period. Cell cultures were incubated in 3 ml of DMEM containing [^{125}I]-LDL (about 0.2 µg of protein) with or without a 500-fold excess of unlabeled LDL for the determination of nonspecific binding. At the end of the incubation period, culture media were collected, and the cell sheets were washed twice with cold saline solution. After cellular digestion with 1N sodium hydroxide, the cell radioactivity was measured. The degradation of [^{125}I]-LDL was evaluated as follows: 2 ml of cell culture medium was mixed with 0.2 ml of plasma before the addition of 0.5 ml of a 50% trichloroacetic acid solution. After 30 minutes at 4° C, proteins were collected by centrifugation. Then 20 µl of a 40% potassium iodide solution and 100 µl of 25% hydrogen peroxide were added to the supernatant. After a 5-minute incubation at 21° C, the free iodine was extracted with chloroform and the radioactivity was counted in 1.5 ml of the aqueous phase. Binding studies of [^{125}I]-LDL were performed at 4° C during an 8-hour period. Nonspecific binding was evaluated by adding an excess of unlabeled LDL. At the end of the incubation time, cell sheets were washed twice with cold saline solution and the radioactivity attached to the cells was measured in the 1N sodium hydroxide–cell digests.

Results

In synthetic medium (DMEM) alone, there was no detectable secretion by JEG-3 cells of dehydroepiandrosterone, testosterone, estrone, and estradiol (controls shown in Fig. 1). Also undetectable (<50 pg/mg of cellular protein per 24 hours) was the secretion of 17-hydroxyprogesterone (data not shown). In contrast, Figs. 2 and 3 show significant secretion of both progesterone (about 15 ng/mg of cell protein per 24 hours) and pregnonolone (about 5 ng/mg of cell protein per 24 hours).

Fig. 1 shows that the addition of DHEA-S between 5 and 50 ng/ml of DMEM induced a dose-related secretion of estrone and estradiol. At higher concentrations of DHEA-S, there was a plateau in the secretion of estrone and estradiol, at about 3 and 30 ng/mg of cell protein per 24 hours, respectively. Concentrations of DHEA-S lower than 50 ng/ml did not elicit a secretion of C-19 steroids; addition of DHEA-S above 50 ng/ml and up to 5 µg/ml induced a dose-related secretion of dehydroepiandrosterone, androstenedione, and testosterone. Fig. 4 shows the inability of DHEA-S (up to 20 µg/ml of DMEM) to affect the basal (de novo) secretion of progesterone. Although the basal secretion of progesterone was unaffected by the addition of DHEA-S, a significant stimulation of pregnenolone secretion was observed (Fig. 3, *left panels*).

The addition of LDL to DMEM resulted in a dose-related increase in progesterone secretion; a twofold to threefold stimulation of progesterone was achieved with LDL concentrations ranging between 0.6 and 3.2 mg/ml of DMEM (Fig. 2). Addition of LDL to DMEM

had no significant effect on the secretion of 17-hydroxyprogesterone or on the secretion of C-19 or C-18 steroids, the levels of which remained undetectable; also unaffected by the addition of LDL was the conversion of DHEA-S into androgens and estrogens (data not shown). The stimulation of progesterone secretion induced by LDL was abolished by the addition of 5 μg/ml of DHEA-S (Fig. 4). While suppressing the LDL-induced progesterone secretion, the addition of DHEA-S resulted in a proportional increase in pregnenolone secretion (Fig. 3, *right panels*).

The possibility that the inhibitory effect of DHEA-S on LDL-stimulated progesterone secretion resulted from an alteration of LDL binding to JEG-3 cells was evaluated at 4° C. Binding equilibrium was reached after 5 hours of incubation, and the fraction of specifically bound [^{125}I]-LDL from the total radioactivity added per culture flask (132,098 ± 1361 cpm) was not significantly different in the absence (1912 ± 214 cpm) or the presence (2185 ± 151 cpm) of DHEA-S (5 μg/ml) in the culture media. At a concentration that abolishes the stimulatory effect of LDL on progesterone secretion, DHEA-S had no significant effect on (1) the specific uptake of [^{125}I]-LDL by JEG-3 cells (Fig. 5) or (2) the quantity of specifically degraded LDL extracted from JEG-3 cell media (Fig. 6).

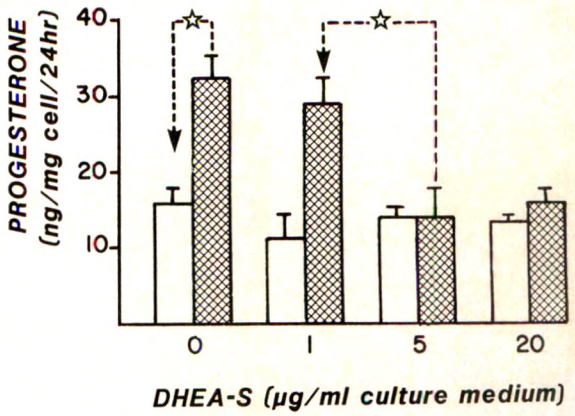


Fig. 4. Effect of DHEA-S on progesterone secretion by JEG-3 cells grown either in DMEM *(open bars)* or in DMEM + 0.7 mg of LDL protein *(hatched bars)*. Remainder as in legend to Fig. 2.

Comment

Like the normal trophoblast, JEG-3 cells have a limited capacity for hormonal storage, and a change in the 24-hour secretion reflects a change in hormonal biosynthesis (Benveniste R, unpublished data). When grown in DMEM alone, JEG-3 cells did not secrete 17-hydroxyprogesterone or C-19 or C-18 steroids; this likely reflects a deficiency in 17-hydroxylase and 17,20-lyase activities, a deficiency also present in the normal human trophoblast.[15] JEG-3 cells are similar to the normal tissue in that they metabolize DHEA-S actively; at physiologic concentrations (0.5 to 5 μg DHEA-S per milliliter), about half of the substrate was converted into dehydroepiandrosterone, androstenedione, testosterone, estrone, and estradiol.

The secretion of estradiol (and that of estrone, which represents about 10% of the secretion of estradiol) was induced by the addition of as little as 5 ng of DHEA-S per milliliter to JEG-3 cell media. Estradiol secretion increased rapidly between 5 and 50 ng of DHEA-S per milliliter but plateaued at higher DHEA-S concentrations. When estradiol plateaued, the secretion of C-19 steroids became detectable and increased with further addition of DHEA-S (Fig. 1). These results are compatible with (1) the high affinity of aromatase(s) for its substrates (Michaelis constant of 10 nmol/L, about 3 ng/ml) as reported in the normal trophoblast[16] and (2) a slow turnover of the aromatase(s) and the rate-limiting effect of that enzymatic system on the conversion of DHEA-S into estrogens.[17]

Progesterone secretion by the trophoblast has been thought to be independent of a fetal adrenal influence as evidenced by a significant progesterone secretion in anencephalic pregnancies, as well as in pregnancies where the fetoplacental circulation is interrupted.[18, 19] In 1981, this notion was challenged by Siiteri and Seron-Ferre.[4] In a study on term placentas perfused with pregnenolone, these investigators reported that about 60% of the progesterone output was suppressed by DHEA-S. Since then, others have shown that DHEA-S has some inhibitory effect and have considered it a possible mechanism by which the fetal adrenal limits the secretion of progesterone by the trophoblast.[20] The relevance of these reports was enhanced by the observation of a marked DHEA-S rise at the time of parturition in primates.[4]

In cultured human choriocarcinoma cells, LDL was shown to inhibit hydroxymethylglutaryl coenzyme A, a key enzyme in the de novo synthesis of cholesterol.[21] The ability of JEG-3 cells to grow in synthetic medium provided a rare opportunity for estimating the de novo progesterone secretion in comparison to the secretion induced by physiologic concentrations of LDL. The secretion of de novo progesterone was very significant, representing 30% to 50% of that achieved by LDL. A similar estimate on the importance of de novo progesterone secretion was obtained from studies on human fetal adrenal cell cultures.[22] It is unclear what advantage cellular steroidogenesis derives from having a complex LDL system coupled to an efficient de novo acetate-cholesterol pathway.

DHEA-S had no significant effect on the de novo secretion of progesterone. In contrast, the increase of progesterone induced by LDL was abolished by DHEA-S (at about 5 μg/ml), bringing the secretion of progesterone back to basal levels (Fig. 4). At levels where DHEA-S inhibited LDL-stimulated progester-

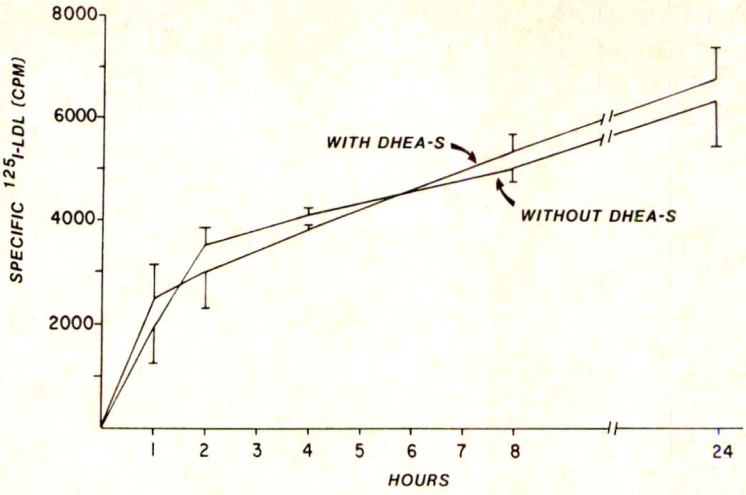


Fig. 5. Effect of DHEA-S (5 μg/ml) on the specific uptake of [^{125}I]-LDL by JEG-3 cells. After 3 days in DMEM culture media were replaced by fresh DMEM containing [^{125}I]-LDL (about 1.5 μg of protein) with or without a 500-fold excess of unlabeled LDL used for the determination of nonspecific binding. Uptake was measured at various times as indicated in Material and methods.

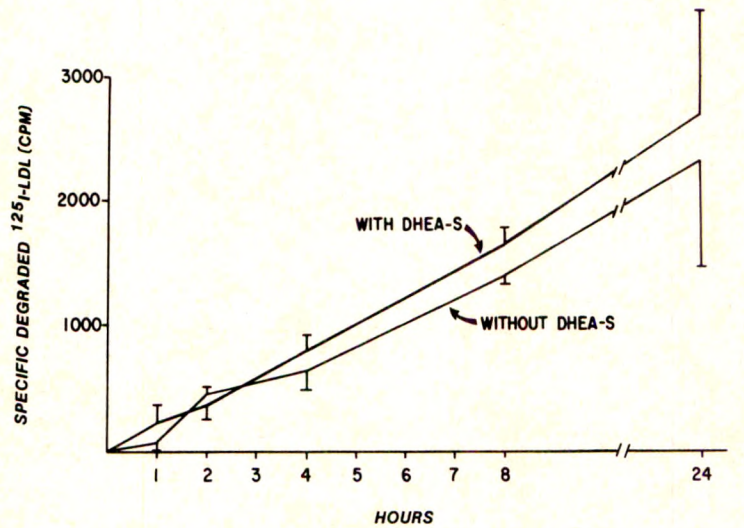


Fig. 6. Effect of DHEA-S (5 μg/ml) on the specific degradation of [^{125}I]-LDL by JEG-3 cells. Culture media corresponding to the experiment shown in Fig. 5 were measured for their content in degraded [^{125}I]-LDL as indicated in Material and methods.

one secretion, estradiol secretion remained maximum. Thus, in JEG-3 cells, a marked increase in DHEA-S is effective in altering the progesterone-estrogen secretory ratio. The differing effects of DHEA-S seen in the present study on basal versus LDL-stimulated progesterone secretion may explain the variable results with DHEA-S reported by others.[4, 20, 23] Indeed our results suggest that the response to DHEA-S would vary depending on the content of serum (or other carriers of preformed cholesterol) in the medium of the experimental model.

The possibility was first considered that ≥5 μg/ml of DHEA-S interferes with the incorporation of LDL by JEG-3 cells. Results showed that DHEA-S had no significant effect on LDL binding, uptake, or degradation (Figs. 5 and 6). Next, the inhibitory effect of DHEA-S on the conversion of pregnenolone into progesterone was considered. A marked increase of pregnenolone secretion was observed after the addition of LDL, and this increase was potentiated by DHEA-S. The effect of DHEA-S in increasing pregnenolone secretion was similar in magnitude to its inhibitory effect on progesterone (Fig. 3), suggesting that in JEG-3 cells exposed to LDL, DHEA-S inhibits 3β-hydroxysteroid dehydrogenase/Δ4,5 isomerase activity and the conversion of prenenolone into progesterone. This notion is consistent with studies on placental homogenates indicating that dehydroepiandrosterone (which is readily formed

by JEG-3 cells) competitively inhibits the conversion of pregnenolone into progesterone.[24] DHEA-S also induced a significant increase of pregnenolone in JEG-3 cells grown in DMEM alone. However, under this latter culture condition, the secretion of progesterone formed de novo was unaffected (Fig. 3). This may reflect a compensatory increase in de novo biosynthesis that follows the DHEA-S inhibitory effect on the conversion of pregnenolone into progesterone, a speculation that deserves further study.

An active form of 3β-hydroxysteroid dehydrogenase/Δ4,5 isomerase is required for the formation of both androstenedione and progesterone. In JEG-3 cells, low DHEA-S concentrations led to a secretion of C-19 and C-18 steroids, while high DHEA-S concentrations (>1 μg/ml) were needed in order to effect LDL-stimulated progesterone secretion. This suggests that in intact JEG-3 cells, 3β-hydroxysteroid dehydrogenase/Δ4,5 isomerase is coupled to two distinct functional units: one responding readily to DHEA-S and involved in androstenedione formation and a second unit affecting progesterone formation and accessed only by high DHEA-S concentrations. Lieberman et al.[25] have recently written on the cell, "hormonads," those functional units "in which reside all the components, enzymes, co-factors, etc. that are required to convert a sterol precursor to a specific hormonal product." In cells of trophoblastic origin, it is possible that 3β-hydroxysteroid dehydrogenase/Δ4,5 isomerase is located within at least two separate "hormonads," on which depends the respective control by DHEA-S of estrogens and progesterone secretion.

In summary, the exposure of JEG-3 cells to low DHEA-S concentrations establishes a basal progesterone-estrogen secretory ratio that is stable within a wide concentration range of DHEA-S. The ratio stability results from (1) a high sensitivity and rapid saturation of the enzymatic system leading to the secretion of estrogens and (2) a relatively high de novo progesterone secretion, which is unaffected by DHEA-S. Physiologic LDL concentrations increase the progesterone-estrogen secretory ratio twofold to threefold, and this increase is obliterated by raising DHEA-S to high concentrations.

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The length of the human umbilical cord in vertex and breech presentations

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The present study is based on two previously stated theories: (1) that the umbilical cord length is influenced by fetal motor activity and (2) that the breech presentation is associated with low fetal motor activity. From this one would expect the mean cord length to be shorter in breech presentations as compared with vertex. This was indeed found to be the case. An already known difference between the sexes regarding cord length was also confirmed, and a difference in male:female ratio in the two presentations is shown. The results of the study thus seem to support the two stated theories. (Am J Obstet Gynecol 1986;154:1086-7.)

Key words: Umbilical cord, vertex presentation, breech presentation

Recent research has suggested that the length of the human umbilical cord at birth is influenced by the intrauterine level of fetal activity.[1,2] The theory submitted states that linear cord growth occurs in response to tensile forces placed upon it.

Tompkins[3] assumed in his article that decreased fetal activity could be associated with breech presentation, although the evidence for this seems unclear.

If these two theories were to be verified, one would expect the length of the cord to be shorter in breech presentation as compared with vertex presentation. This study was undertaken to investigate if this difference exists.

Numerous articles have been published on the length of the human cord,[4-9] and various attempts have been made to correlate the cord length to other fetal or maternal data. Of these, only two seem to have concerned themselves with the problem of the presentation. Gardiner[4] stated that the cord must be longer in breech presentations than in vertex presentations if uncomplicated birth is to occur. However, he did not present any empirical data to support his assumption.

Malpas[6] included in his material of 538 cords only four associated with breech presentation, and he made no attempt to draw inferences from this small number.

Extensive studies have tried to correlate various maternal and fetal factors to the labor presentation.[10-14] None of these have taken the umbilical cord length into account. Those factors that were supposed to differ between breech and vertex presentations (gestational age, fetal weight) have been shown earlier not to correlate[5] or to correlate only slightly[6,8] with umbilical cord length.

To the best of our knowledge no other studies so far have been published on this subject.

Table I. Data on mean cord length

	Vertex	*Breech*	*Difference*
Mean length			
Male infants	58.46	53.78	4.68
Female infants	56.90	52.51	4.38
Differences	1.56	1.26	—

Material and methods

Between 1979 and 1983, a total of 10,887 births were recorded in our department. This figure includes both vaginal and cesarean deliveries. Stillbirths and premature deliveries as early as 28 weeks of gestation are included. We felt that this could be done confidently since previous studies have not shown any significant linear cord growth after 28 weeks of gestation.[5,6]

For the designation vertex presentation only the occipitoanterior presentation is included. All other variations of the vertex presentation are excluded. The term breech presentation includes all variations of breech. Transverse lie has been excluded. Multifetal pregnancies have been excluded.

A total of 9899 births fell into the two categories. Umbilical cords were measured in 9601 of these. In our hospital the cord is routinely cut 2 cm from the fetal abdomen. The length is then measured from this point to the placental surface.

Results

The relevant data on mean cord length are presented in Table I. All lengths are given in centimeters.

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We have presented the two sexes separately. Walker and Pye[5] found that male infants had somewhat longer umbilical cords than female ones. This result is consistent with our findings in that the difference was 1.56 cm in the vertex presentations and 1.26 in the breech ones (only the former of these is significant, $p < 0.01$). Furthermore it is shown that the male:female ratios were significantly different in the two presentations ($p < 0.01$). In the vertex presentation, the male:female ratio was 1.08, which is consistent with generally accepted figures. In the breech presentation the male:female ratio was 0.85.

The distribution of the cord length was nonnormal, with a bimodal shape. This is consistent with the results of Malpas[6] and Fog[9] and is shown by the latter to be caused by the summation of two different populations: umbilical cords with coils around the fetal body and cords without any coils. The former tend to be somewhat longer. No distinctions have been made between coiled and noncoiled cords in this study.

Comment

The results show a difference between mean umbilical cord length in the vertex versus the breech presentation of approximately 4.5 cm. This supports the theory that fetuses with decreased levels of motor activity also have a tendency to assume the breech presentation at term. Whether this decreased activity is caused by some fetal factor or by intrauterine constraint is at the moment not clear. One could assume that both factors are important.

The findings that male fetuses have a slightly longer cord than female fetuses and that female fetuses tend to assume the breech presentation more often than male fetuses are also consistent with the general theory. It is, however, difficult to imagine that any external factor such as intrauterine constraint produces this effect. It seems more likely that fetal sex has a direct influence on the fetal level of spontaneous activity. On the other hand, a hitherto unknown sex-related mechanism acting directly on cord growth cannot be excluded.

A possible relation between coiling of the cord and the fetal presentation remains unknown. The sex difference in fetal motor activity, which is assumed in this article, needs further investigation. The ultimate goal would be to shed some light upon the unknown mechanisms that cause the breech presentation.

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The tumor marker CA 125 is a common constituent of normal cervical mucus

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The presence of the tumor marker CA 125 was studied in the cervices of healthy women. Immunohistochemical staining of normal cervical tissue demonstrated the presence of CA 125 in the tall columnar cells of the endocervical epithelium but not in the ectocervical squamous epithelium. We measured very high levels of CA 125 in liquefied cervical mucus from women with regular menstrual cycles. At midcycle, levels ranged from 14,200 to 153,000 U/ml (n = 13) in cervical mucus, while normal levels <35 U/ml were found in the corresponding serum samples. Levels of CA 125 in cervical mucus are comparable to the high levels found in cyst fluids from ovarian tumors (median 24,600 U/ml, n = 25). When secretion of cervical mucus was stimulated by ethinyl estradiol, equally high levels were found (7900 to 138,000 U/ml, n = 10). We conclude that the tumor marker CA 125 is synthesized and secreted by normal endocervical cells. Apparently an effective barrier exists between the endocervical mucosa and the circulation. (Am J Obstet Gynecol 1986;154:1088-91.)

Key words: CA 125, cervical mucus, ovarian tumor marker

CA 125 is an antigenic determinant produced by nonmucinous epithelial ovarian tumors and has been proposed as a tumor marker for patients with ovarian cancer.[1, 2] Its identity is defined by the interaction with the murine monoclonal antibody OC 125.[3] Increased serum levels of CA 125 were found in 90% of patients with manifest epithelial ovarian cancer, and rising or falling serum levels were associated with tumor progression and regression, respectively. Some patients with adenocarcinoma of the cervix or the endometrium also showed raised levels of serum CA 125.[4] It was proposed that CA 125 is a differentiation antigen, shared by fetal coelomic epithelium and its derivates in the adult, which is reexpressed in neoplastic ovarian epithelial cells.[5]

We found high levels of the antigen CA 125 in normal cervical mucus from ovulatory women and confirmed its presence in the columnar cells of the normal endocervix by immunohistochemical methods.

Methods

Patients. Twenty-three women who were patients of the Fertility Department of the University Hospital, Groningen, were examined. Thirteen women with normal menstrual cycles, biphasic basal body temperature curves, and normal viscoelastic properties of the cervical mucus were selected. Their ages ranged from 20 to 41 years. The cervical mucus samples were obtained at midcycle before ovulation, The mucus samples from 10 other women were obtained in the second week of a stimulated pill cycle. These women were prescribed 50 μg of ethinyl estradiol per day from the third to the fifteenth day after beginning of the menses. The cervical mucus was aspirated from the endocervix with the use of a long tuberculin syringe without needle. All cervical mucus samples investigated showed signs of good estrogenic stimulation. The cervical mucus samples were liquefied by sonication for 1 minute by an MSE Sonificator with a small tip. Debris was removed by centrifugation at 2000 × g for 5 minutes. The supernatants were stored at −20° C.

On the day of the cervical mucus collection blood samples were drawn from 19 of the 23 women. Blood samples were also collected from 13 women with chronic cervicitis. These women had a longer history of mucopurulent discharge from the external cervical os or contact bleeding. The diagnosis was confirmed by microscopic examination of colposcopically directed punch biopsies and material from endocervical scraping.

As a comparison CA 125 was measured in tumor cyst fluid samples and blood samples from 32 patients with epithelial ovarian tumors. These samples were collected before any treatment was started and they were centrifuged at 2000 × g for 5 minutes. The supernatants were stored at −20° C.

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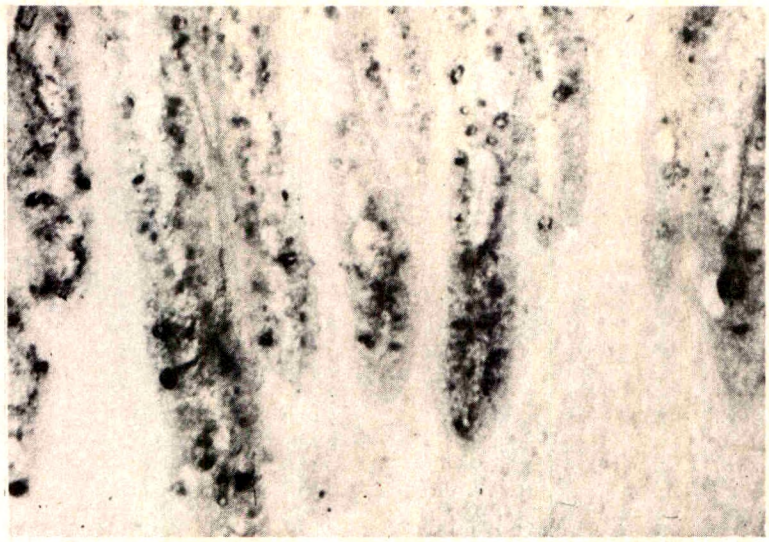


Fig. 1. Light microscopic picture of a frozen section of the endocervix incubated with monoclonal antibody OC 125. An intense staining of the endocervical cells is present. (Indirect peroxidase. Original magnification ×250.)

Measurement of CA 125. Levels of CA 125 in cervical mucus, cyst fluids, and serum were assayed with a simultaneous sandwich immunoradiometric assay as described by Bast et al.[1] The reagents were obtained from Centocor Inc. (Malvern, Pennsylvania). The extremely high levels of CA 125 in undiluted samples of cervical mucus gave an excess of antigen that blocked the iodine 125–labeled antibody, resulting in a strongly diminished binding. Therefore, multiple dilutions always up to 1/1000 were assayed for CA 125. Dilutions were made with a standard serum diluent.[1] Control measurements were done by incubation of cervical mucus samples in a two-step incubation IRMA, as described by Klug et al.[6] In this procedure the excess of antigen was removed by washing after incubation of the sample, and a separate incubation with the labeled antibody followed. All measurements were done in duplicate. Results were expressed as units per milliliter. A sensitivity of 9 U/ml was obtained. For serum samples an upper limit of normal was chosen at 35 U/ml.[1] A control serum sample was measured in 14 different assays and a variation coefficient of 8.5% was observed.

Immunohistochemistry. Endocervical tissue was obtained from autopsy or from surgical specimens of eight patients with benign or nongynecologic diseases. Their ages ranged from 13 to 87 years. CA 125 was demonstrated in snap-frozen cervical tissue blocks by the indirect peroxidase–peroxidase method essentially the same as described previously.[7] In brief, the following incubations were used: (1) Incubation was performed for 60 minutes with monoclonal antibody OC 125 (gift from Oris Lapam, France). (2) The sections were incubated with horseradish peroxidase–conjugated rabbit antimouse serum (Dakopatt, Denmark) for 30 minutes. (3) Development of peroxidase activity was performed with a freshly prepared solution of 3-amino-9-ethylcarbazole in 0.1 mol/L acetate buffer, pH 5.0, containing 0.03% hydrogen peroxide resulting in a red reaction product. Between incubations sections were washed three times for 10 minutes in phosphate-buffered saline, pH 7.4. Finally, sections were counterstained with hematoxylin for 2 minutes and mounted in Kaises glycerine gelatine (Merck, Germany).

Results

Immunohistochemical staining of normal cervical tissue demonstrates the presence of CA 125 in the tall columnar cells of the endocervical epithelium (in all eight tissue samples investigated). The staining was most intense at the luminal surfaces and the apical portion of the cytoplasm (Fig. 1). The ectocervical squamous epithelium was completely negative.

High levels of the antigen CA 125 were found in liquified cervical mucus from women with regular menstrual cycles. At midcycle, the levels ranged from 14,200 to 153,000 U/ml (Table I).

Women who received ethinyl estradiol produced a thin and clear cervical mucus with equally high levels (7900 to 138,000 U/ml). Control measurements in the two-step incubation procedure confirmed the presence of the high CA 125 levels in the mucus and an acceptable correlation was obtained ($r = 0.869$, $p < 0.001$). The level of CA 125 in cervical mucus was of the same order of magnitude as that found in cyst fluid samples derived from benign and malignant epithelial ovarian tumors (Table II).

Table I. Midcycle CA 125 levels in cervical mucus and serum from women with regular menstrual cycles

No.	*Cervical mucus (U/ml)*	*Serum (U/ml)*
Natural cycle		
1	66,500	18
2	27,800	31
3	116,000	30
4	117,000	23
5	75,700	21
6	108,000	21
7	153,000	20
8	104,000	–*
9	14,200	–
10	16,800	20
11	73,700	23
12	59,300	–
13	26,400	–
Mean	73,700 ± 44,200	
Ethinyl estradiol stimulated		
14	109,000	26
15	84,600	22
16	109,000	24
17	138,000	23
18	30,000	22
19	7,900	35
20	60,800	30
21	11,400	26
22	16,600	13
23	21,700	23
Mean	58,900 ± 48,100	

*No serum sample available.

Table II. Median levels of CA 125

	No.	*Median CA 125 (U/ml)*	*Range*
Cervical mucus from preovulatory women	23	65,500	7,900-153,000
Cyst fluids from epithelial ovarian tumors	25	24,600	770-2,140,000
Serum from patients with epithelial ovarian cancer before treatment	32	424	11-4,480
Serum from healthy women	19	<35	13-35
Serum from patients with chronic cervicitis	13	<35	10-69

In serum, levels of CA 125 were below the limit of 35 U/ml in all 19 women investigated at the time of cervical mucus aspiration (Table I). Only one of 13 patients with chronic cervicitis demonstrated an elevated CA 125 level of 65 U/ml (Table II). In a control experiment we confirmed the presence of elevated CA 125 levels in preoperative serum samples from patients with epithelial ovarian cancer; in 30 of 32 patients levels >35 U/ml were seen.

Comment

Tumor antigens are used as markers in clinical oncology mainly for immunohistochemical diagnosis and monitoring of the effect of therapy. These antigens often occur in normal cells and are, so far, not tumor specific. In the search for specific antigens from ovarian cancer a monoclonal antibody OC 125 was developed that reacted with a cell line from an ovarian cystadenocarcinoma.[3] The undefined antigen was termed CA 125, and to date CA 125 has been characterized only as a high molecular weight glycoprotein.[1] Our study demonstrates the presence of CA 125 in cervical mucus and we conclude that CA 125 is a secretory product of normal cells and not a tumor-specific antigen. The production site of the CA 125 is, at least in part, the columnar endocervical epithelium, since the presence of CA 125 in the epithelial cells could be demonstrated immunohistochemically. The columnar epithelium of the endocervical canal and its diverging crypts are known to produce and secrete glycoproteins. These cells show secretory granules and irregular vacuolization characteristic of the mucin cell type. They stain deeply red with the periodic acid–Schiff method.[8] It cannot be excluded that some of the CA 125 in the cervical mucus originates from other parts of the female genital tract. Presence of CA 125 in cervical mucus due to deposition by semen could be excluded since the antigen could not be detected in seminal plasma or on spermatozoa (unpublished observations).

Despite the high levels in cervical mucus, only small amounts of CA 125 could be detected in serum from normal women. Apparently this is because an effective barrier exists between the endocervical mucosa and the circulation. We investigated serum CA 125 levels in patients with chronic cervicitis because in endocervical biopsy specimens subepithelial infiltration of lymphocytes and plasma cells is often present. This condition may enhance transfer of CA 125 into the circulation. One of the 13 patients studied showed a raised level. Niloff et al.[9] were unable to find elevated serum levels in women with vaginitis or cervicitis. However, they used 65 U/ml as the upper limit of normal. Although CA 125 is not tumor specific, it may be quite useful for monitoring the response to therapy and early detection of tumor recurrence in patients with ovarian epithelial cancer. The specificity of raised serum levels of CA 125 as an indicator of malignancy remains to be assessed.

The presence of large quantities of CA 125 in the normal female genital tract makes it worthwhile to study the functional properties of this antigen.

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Human chorionic gonadotropin in early normal and pathologic pregnancy

Discordant levels in peripheral maternal blood and blood from the uterine and abdominal cavities

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Human chorionic gonadotropin was assayed in 25 cases after first-trimester induced abortion, in 45 cases of spontaneous abortion in the first trimester, and in 27 cases of ectopic pregnancy. Blood was obtained from an antecubital vein and from the uterine cavity. In the cases of ectopic pregnancy blood was also obtained from the abdominal cavity. In the group of induced abortion the human chorionic gonadotropin levels in peripheral maternal blood did not differ significantly from the levels in the uterine cavity. In the groups of spontaneous abortion and ectopic pregnancy the human chorionic gonadotropin levels were significantly higher in blood from the uterine cavity and the abdominal cavity, respectively. In four cases (three with spontaneous abortion and one with an ectopic pregnancy) human chorionic gonadotropin was not detectable in peripheral maternal blood, while it was found in blood from the uterine and abdominal cavities. (AM J OBSTET GYNECOL 1986;154:1091-4.)

Key words: Human chorionic gonadotropin, ectopic pregnancy, spontaneous abortion, induced abortion

Human chorionic gonadotropin (hCG) is produced in large quantities by the syncytiotrophoblast. By use of sensitive radioimmunoassays it is detectable in peripheral maternal serum as early as 6 to 8 days after conception.[1] In early normal pregnancy the hCG levels rise rapidly, with a doubling time of 1.7 to 2 days, reaching a peak after 60 to 80 days.[2] In pathologic conditions such as ectopic pregnancy and spontaneous abortion the levels are found to be lower than in normal gestation of the same duration.[3] Some pathologic cases are on record in which no trace of hCG in peripheral blood was found, although histologic examination of the surgical specimens confirmed pregnancy. Devitalized trophoblast with no circulating hCG has been suggested as the most likely explanation.[4] We have observed pathologic pregnancies in which blood from the uterine cavity and blood from the peritoneal cavity have con-

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Table I. The age and duration of amenorrhea of the examined patients by type of pregnancy termination

Group	*Age, range (yr)*	*Amenorrhea* (wk)*	
		Range	*Mean ± SD*
I. Induced abortion (n = 25)	16-19	7-10	8.6 ± 1.0
II. Spontaneous abortion (n = 45)	15-41	3-14	9.3 ± 2.3
III. Ectopic pregnancy (n = 27)	17-40	3-10	6.1 ± 1.9

*In weeks, counted from the first day of the last menstrual period.

Table II. Four patients with nondetectable hCG levels in peripheral circulation but detectable levels in uterine and abdominal cavities with corresponding histologic findings

	Amenorrhea (wk)	*hCG (U/L)*			*Histologic features*
		Antecubital venous blood	*Uterine cavity blood*	*Abdominal cavity blood*	
Complete spontaneous abortion	6	<5	7.4		Secretory endometrium
Complete spontaneous abortion	4	<5	36		Secretory endometrium
Complete spontaneous abortion	13	<5	48		Decidual reaction
Ectopic pregnancy	8	<5	276	363	Secretory endometrium

Table III. hCG levels and corresponding histologic findings in curettage specimens of three patients with nonruptured fallopian tubes

hCG (U/L)				
Antecubital venous blood	*Uterine cavity blood*	*Intraperitoneal cavity blood*	*Fallopian tube blood*	*Histologic examination*
16	15	3,070	19,900	Secretory endometrium
787	3,460	14,400	52,800	Secretory endometrium
1,130	1,410	17,800	39,400	Secretory endometrium

tained relatively high levels of hCG in the presence of low or no detectable levels in peripheral blood. This could mean that the trophoblast is still vital with continued production of hCG, in amounts too small for detection in peripheral blood. The discordant hCG values in serum specimens from different sources in the same patient were evaluated in this study.

Material and method

Three groups of women were examined. Relevant characteristics are listed in Table I.

The first group consisted of 25 patients admitted to the hospital for legal termination of uneventful first-trimester pregnancy, all terminated by vacuum suction followed by curettage. Blood samples were drawn simultaneously from an antecubital vein and from the uterine cavity after rupture of the membranes and partial removal of the pregnancy products.

The second group consisted of 45 patients admitted because of spontaneous abortion. All had vaginal bleeding and lower abdominal pain with dilatation of the cervical os. In no case did ultrasonography show an intact gestational sac. Forty-two patients had an incomplete abortion and three had a complete abortion. In 39 cases the removed tissue was examined histologically. In the remaining six macroscopic examination revealed undisputable pregnancy products. Blood samples were drawn simultaneously from an antecubital vein and from the uterus or vaginal vault during evacuation.

The third group consisted of 27 patients with ectopic pregnancy. In all cases the pregnancy was located in a fallopian tube, which was ruptured in 24 and not ruptured in three cases. The amount of intraperitoneal blood was measured, and the diagnosis was confirmed by histologic examination of the removed tubal tissue. In all cases blood samples were obtained simultaneously from the intraperitoneal cavity and an antecubital vein. In 13 cases blood samples were also obtained from the uterine cavity during curettage. In the three cases with nonruptured fallopian tubes blood was aspirated by puncture of the affected tube before removal of the pregnancy.

hCG was assayed in serum by an hCG β-subunit ra-

dioimmunoassay method without extraction. The technique is a modification of a method described by Vaitukaitis et al.,[5] which has been described in detail in a previous report.[6] The sensitivity of the method is 5 U/L of hCG.

Results

The diagnoses and the levels of hCG are shown in Fig. 1.

In the patients having induced abortion 14 had higher levels of hCG in the antecubital vein than in the uterine cavity, while 11 had slightly higher levels in blood from the uterine cavity.

Of the patients with spontaneous abortion 42 had significantly higher levels of hCG in blood from the uterine cavity than from the antecubital vein (2 $p < 0.0001$, Wilcoxon test for two samples). The approximate values can be read from Fig. 1. The three patients with complete abortion had no detectable hCG in antecubital vein blood and low values of hCG in blood from the uterine cavity, presented with connecting lines in Fig. 1. The findings in these three patients including the results of the histologic examination are listed in Table II.

In the group of women with ectopic pregnancy the amounts of intra-abdominal hemorrhage ranged between 20 and 1500 ml with a median volume of 100 ml. All 27 patients had significantly higher levels of hCG in blood from the intraperitoneal cavity than in blood from an antecubital vein (2 $p < 0.0001$). Of the 13 patients in whom blood was obtained from the uterine cavity, all had significantly higher levels of hCG in abdominal blood than in blood from the uterus (2 $p < 0.005$). Nine patients also had higher levels of hCG in uterine blood than in blood from the antecubital vein ($p < 0.005$). One patient had hCG amounts of <5, 363, and 276 U/L in antecubital venous blood, abdominal blood, and uterine blood, respectively. Table III gives the results of three patients with nonruptured fallopian tubes. All had much higher levels of hCG in the fallopian tube than in the other serum specimens.

Comment

The main portion of the hCG produced by the trophoblast is secreted into the maternal circulation.

The detection of hCG in peripheral maternal serum has been taken as evidence of vital trophoblast and, conversely, negative tests have been interpreted as indicating no pregnancy present. Quantitative measurements with sensitive radioimmunoassays have revealed lower levels of hCG in peripheral maternal serum in cases of pathologic pregnancies such as spontaneous abortions and ectopic pregnancies as compared with those of normal gestations of the same duration.[3]

The current results correlate well with this, but they also reveal a significant difference between the low levels of hCG in peripheral maternal blood and the higher levels in blood from the site of gestation in cases of pathologic pregnancies. This contrasts with the high values of hCG in blood from both the antecubital vein and the uterine cavity in normal pregnancies. It might also rule out a simple dilution effect in the maternal circulation as the only explanation of this discordance.

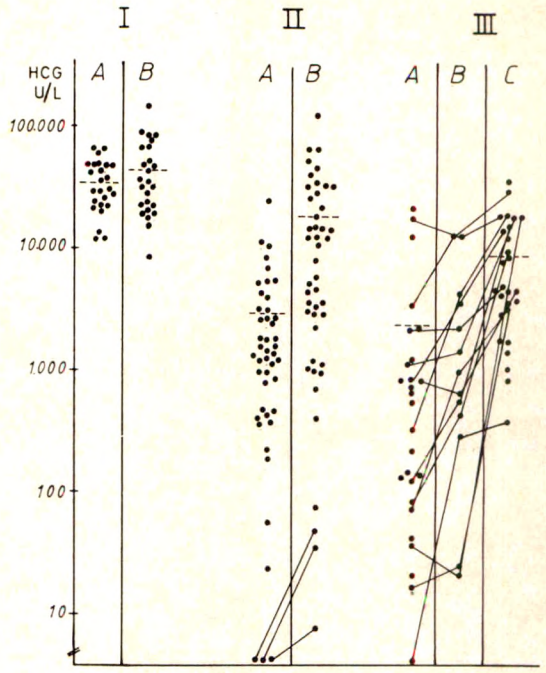


Fig. 1. The hCG levels in blood from an antecubital vein *(A)*, from the uterine cavity *(B)*, and from the abdominal cavity *(C)* in patients with induced abortion *(I)*, spontaneous abortion *(II)*, and ectopic pregnancy *(III)*. The connecting lines in group *II* represent the three patients with nondetectable hCG values in an antecubital vein and the corresponding hCG values in blood from the uterine cavity. In group *III* they represent the 13 patients in whom hCG was measured in the three specimens.

Sandvei et al.[4] found nondetectable levels of hCG in three of 100 cases of ectopic pregnancy with devitalized pregnancy tissue. In our study one patient with ectopic pregnancy and three with spontaneous abortions had no detectable hCG (<5 U/L) in peripheral maternal serum while still having positive values of hCG in blood from the uterine cavity (two patients) and abdominal cavity (three patients) (Table II).

The high levels of hCG in blood from the nonruptured fallopian tubes are not surprising, since the tubes are so near the site of production (Table III).

Earlier studies have revealed a long time until disappearance of hCG after termination of normal pregnancies and a much shorter time span after removal of ectopic pregnancies and spontaneous abortions as the result of a combination of factors including the pro-

duction and elimination of hCG.[6] In cases of ectopic pregnancy partial devitalization of the placenta is reported.[4] At least some devitalization of pregnancy tissue would also be expected in spontaneous abortions, and in both conditions this could result in less circulating hCG. Reduced blood flow and small functioning areas of the placenta could theoretically also involve qualitatively different secretory activity of the trophoblast, resulting in altered forms of the hCG molecule. In support of this theory a shorter plasma half-life and altered immunoreactivity produced by free subunits, peptide fragments, desialated or asialo hCG, and modified carbohydrate residues of hCG have been reported.[7-11]

Also a large molecular species which interacts with antisera to hCG, α-subunit hCG, and β-subunit hCG has been described.[12]

Another possibility for the discordant hCG values obtained could be the peripheral catabolism, which in addition to secretion might play an important role in regulating the serum level of biologically active hormone.[13]

The present findings raise questions that need further investigation, some of which concern the molecular basis of hCG. This involves the problem of different antibody specificity for different molecular forms of hCG.

As a practical conclusion, when a sensitive radioimmunoassay is used for detection of hCG, a negative test in peripheral maternal blood does not always exclude pregnancy. Blood obtained from the intraperitoneal cavity or from the vagina should also be examined for hCG as a further step to confirm or exclude pregnancy.

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Factors related to the increasing cesarean section rates for cephalopelvic disproportion

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At the Highland Park Hospital the increase in cesarean section rates has paralleled the national increase. Data from cesarean deliveries performed for cephalopelvic disproportion (a leading reason for cesarean section) in 1969 to 1972 (group 1) and 1979 to 1982 (group 2) were evaluated to determine maternal, fetal, and delivery factors that might be related to the increase in the rates. The data suggested that the more frequent diagnosis of cephalopelvic disproportion and the increased size of the group 2 infants were the primary reasons for the increase in cesarean section rate. This increased rate was paralleled by a decline in forceps-assisted delivery and in perinatal mortality rates. (AM J OBSTET GYNECOL 1986; 154:1095-8.)

Key words: Cesarean section, cephalopelvic disproportion, fetal weight

During the past 20 years considerable change has occurred in obstetric practice. The widespread use of electronic intrapartum and prenatal fetal monitoring and the use of other surveillance methods to assess fetal well-being have been paralleled by an increase in cesarean section rates. Numerous studies have been undertaken to evaluate factors associated with the increasing cesarean section rates. In 1980, a National Institutes of Health Consensus Development Conference was held to address issues related to cesarean childbirth.[1] At this conference it was reported that in the United States the cesarean section birth rate per 100 births increased from 5.7 in 1970 to 14.7 in 1978. Dystocia was found to be one of the leading indications for cesarean section and contributed to 30% of the increase in the cesarean section rates from 1970 to 1978.

At the Highland Park Hospital the increase in cesarean section rates is similar to the national increase. The cesarean section rate increased from 5.4 per 100 deliveries in 1969 to 1972 to 13.1 per 100 deliveries in 1979 to 1982. At the same time there was a significant decrease in the proportion of forceps-assisted deliveries, from 25.6 per 100 deliveries in 1969 to 1972 to 6.3 per 100 deliveries in 1979 to 1982 (Table I). It is my hypothesis that the increase in cesarean section rates during the 10-year period is due primarily to the more frequent diagnosis of cephalopelvic disproportion as an indication for cesarean section. In these cases, attempts at difficult midforceps vaginal deliveries might compromise the infants, and cesarean sections are more frequently performed.

The purpose of the present article is to evaluate factors that might be associated with the increasing rate of cesarean sections for women with cephalopelvic disproportion who were delivered of their infants at the Highland Park Hospital.

Material and methods

The Highland Park Hospital is a private community hospital in north suburban Chicago. In recent years about 900 deliveries a year have been performed at the hospital. To evaluate factors that might have contributed to the rising cesarean section rate for cephalopelvic disproportion, the medical records of women who were delivered of their infants by cesarean section in 1969 to 1972 and in 1979 to 1982 were identified. These medical records were reviewed to identify women who had cesarean sections performed for indications in the cephalopelvic disproportion spectrum. A total of 186 women were identified, 48 who were delivered in 1969 to 1972 (group 1) and 138 who were delivered in 1979 to 1982 (group 2). Excluded from both groups were multiple gestations, deliveries of infants who weighed <1500 gm at birth, and women with indications for cesarean section other than cephalopelvic disproportion. Lumbar epidural analgesics and anesthetics were not used in either group. There was no significant difference in the pattern of analgesics or anesthetics between the two groups.

The following data were abstracted from the medical records of group 1 and 2 subjects: maternal demographic characteristics, maternal weight gain, maximum dilatation reached before delivery, time from maximum dilatation to delivery, maximum station

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Table I. Cesarean section (all indications) and forceps-assisted delivery rates

Year	*No. of deliveries*	*Cesarean sections (%)*	*Forceps-assisted deliveries (%)*	*Midforceps-assisted deliveries (%)*
1969-1972	3136	5.4	25.6	2.28
1979-1982	3598	13.1	6.3	0.87

Table II. Rates of cephalopelvic disproportion

Time period	*No. of deliveries*	*Cesarean sections (%)*	*Cephalopelvic disproportion*	
			% of Cesarean sections	*% of Deliveries*
1969-1972	3136	5.4	28.3	1.5
1979-1982	3598	13.1	29.2	3.8

Table III. Maternal characteristics

Characteristic	*Group 1*	*Group 2*
Age (%)		
<25 yr	33	17
25-34 yr	67	75
>34 yr	0	8
Mean age (yr)	25.4	29.3
Primiparous (%)	77	88
White (%)	95	92
Average weight gain (pounds)	23	28
Weight gain >25 pounds (%)	29	65

Table IV. Maximum cervical dilatation before cesarean section

Maximum dilatation (cm)	*Group 1 (n = 48) (%)*	*Group 2 (n = 138) (%)*
0-2	20.8	5.1
3-5	33.3	23.9
6-7	16.6	13.0
8-10	29.3	58.0

reached, persistent occipitoposterior position, time of day of delivery, use of Pitocin, 1- and 5-minute Apgar scores and fetal weight. A woman's weight gain was determined as the difference between weight at hospital admission and weight at the time pregnancy was diagnosed. These data were analyzed to evaluate differences between maternal characteristics, events relating to labor, and fetal factors in group 1 and 2 subjects that might relate to the increased cesarean section rate. The data were analyzed by use of standard statistical methods for continuous and categorical data. In the text, only the p values (significance levels) associated with the tests are given.

Results

Similar ($p > 0.10$) proportions (about 29%) of subjects who underwent cesarean section in 1969 to 1972 and 1979 to 1982 were diagnosed as having cephalopelvic disproportion (Table II). However, when considered as a proportion of deliveries there was a significant increase ($p < 0.01$) from 1969 to 1972 to 1979 to 1982 in the percentage of subjects with cephalopelvic disproportion who had cesarean sections.

Maternal factors. Maternal factors for group 1 and 2 subjects are given in Table III. Compared with the group 1 subjects, the group 2 subjects were older ($p < 0.05$) and gained more weight during pregnancy.

Factors related to delivery. The maximum cervical dilatation achieved before cesarean section was greater for group 2 than for group 1 subjects ($p < 0.05$) (Table IV). The mean time at the maximum cervical dilatation achieved was about 30 minutes longer for the group 2 subjects (3.0 versus 2.5 hours). Also the maximum station of fetal head descent was greater ($p < 0.05$) for the group 2 subjects. In group 1 the fetal head did not engage the vertex in 46% of the subjects. The corresponding rate for the group 2 subjects was 30%. The rates of Pitocin augmentation were similar ($p > 0.10$) for the two groups (group 1, 27%; group 2, 28%). The rates of persistent occipitoposterior presentations were similar ($p > 0.10$) for groups 1 and 2 (31% versus 22%).

The time of day when cesarean section was performed was evaluated to determine if there were particular periods during the day when the procedures were more likely to be performed. For group 1 subjects, the procedures were uniformly distributed throughout the day. For group 2 subjects, there was a decrease ($p < 0.05$) in the number of procedures performed from midnight to 4 AM compared with 8 PM to midnight.

Fetal factors. The average 1- and 5-minute Apgar scores were similar ($p > 0.10$) for the two groups (group 1, 9 and 10; group 2, 8 and 9). One infant (2%) in group 1 and nine infants (7%) in group 2 had 1-minute Apgar scores of <7 ($p > 0.10$). All 5-minute Apgar scores were ≥7.

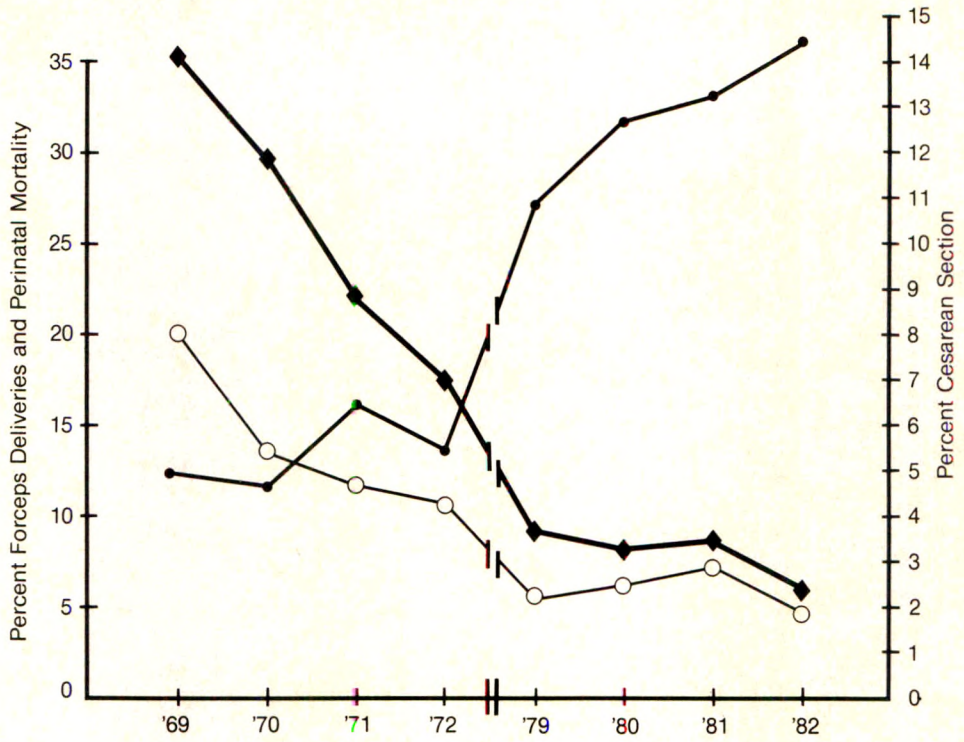


Fig. 1. Comparison of perinatal mortality rates, forceps-assisted delivery rates, and cesarean section rates, 1969 to 1972 and 1979 to 1982. ♦, Forceps rate; ●, cesarean section rate; ○, perinatal mortality rate.

There were too few perinatal deaths in groups 1 and 2 for reliable assessment of perinatal mortality rates. For all deliveries, the perinatal mortality rates (per 1000 deliveries) decreased from 14 in 1969 to 1972 to seven in 1979 to 1982. This reduction in the perinatal mortality rates was paralleled by a decrease in the rate of forceps-assisted deliveries and an increase in the cesarean section rate (Fig. 1).

The mean birth weight of group 2 infants (3772 gm) was significantly higher ($p > 0.05$) when compared with the mean birth weight of group 1 infants (3486 gm). However, the mean birth weights of infants were delivered vaginally during the same time periods (1969 to 1972 and 1979 to 1982) were similar ($p > 0.10$) (3402 versus 3430 gm). The mean birth weights of all infants born at the Highland Park Hospital during the two time periods were 3430 and 3570 gm, respectively. The corresponding birth weights for all infants born in the United States in 1972 and 1982 were 3300 and 3363 gm (United States Bureau of Statistics).

Comment

Although there were some differences between the group 1 and 2 subjects and factors related to their deliveries, none of the factors evaluated, considered either individually or as a group, explained the higher cesarean section rate for deliveries in 1979 to 1982 compared with 1969 to 1972. In fact, the data suggest that the group 1 patients were more aggressively managed (shorter second stage of labor, less cervical dilatation at the time of cesarean section) than the group 2 patients. The data show that a more cautious attitude was taken by obstetricians in later years toward performing cesarean section for cephalopelvic disproportion. The only fetal factor evaluated that could explain the higher cesarean section rate for the group 2 subjects was their greater average weight.

The larger infants of the group 2 mothers may be related to the greater weight gain of their mothers. The recent practice of discouraging women from smoking during pregnancy and improved nutrition could also account for the larger infants. However, no data on parental smoking habits or maternal nutrition were available for evaluation. Since the differences in the mean birth weights of all infants (at Highland Park Hospital and in the United States) and those delivered vaginally during 1969 to 1972 and 1979 to 1982 were small, it is reasonable to assume that none of the differences between the group 1 and group 2 subjects accounted for the greater weight of the infants of group 2 mothers. The most likely explanation for the increased cesarean section rates is that cephalopelvic disproportion was diagnosed with increased frequency as an indication for cesarean section.

Hughey et al.,[2] in 1977, suggested that the increased cesarean section rate due to dystocia was a result of

increased birth weight. These investigators noted that the average birth weight increased by 100 gm during a 6- to 8-year period when cesarean section rates were increasing. Data from New York[1] have shown the increased rate of cesarean sections to be similar across birth weights. However, data that gave the cesarean section rates for different indications across weight classes were not provided. Without these data, one cannot discount the hypothesis that the increased cesarean section rate is due primarily to the more frequent diagnosis of cephalopelvic disproportion and secondarily to the larger infants. Further study of this issue is required. If part of the increased rate of cesarean section is due to the increased frequency of diagnosis of cephalopelvic disproportion, then this part of the increased rate is justified. It may lead to lower perinatal mortality rates and fewer complications in the infants.

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Effects of maternal transport on admission patterns at a tertiary care center

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Major changes have occurred in the delivery of perinatal care during the last two decades. Documentation of perinatal regionalization statistics at Women and Infants Hospital of Rhode Island in Providence support (1) the increasing proportion of low birth weight infants in the region being born at the tertiary care center, (2) the decreasing number of neonatal transports in concert with an increasing number of maternal transports, and (3) the increasing proportion of neonatal transports with birth weights ≥2500 gm. Patterns of modern perinatal care are materially changing the delivery of health care at tertiary care facilities. (Am J Obstet Gynecol 1986;154:1098-100.)

Key words: Perinatal regionalization, maternal transports, neonatal transports

During the past two decades, major changes have occurred in the delivery of perinatal care. Since the advent of perinatal regionalization,[1] there has been a trend of neonatal transports from community hospitals to tertiary centers associated with significant decreases in neonatal morbidity and mortality rates.[2, 3] During the past decade increasing numbers of maternal transports have also been reported.[3] The latter may affect utilization patterns at tertiary care centers. We speculated that as the maternal transport rate rose, the neonatal transport rate would fall, since mothers with high-risk pregnancies would have been transported antenatally and would be delivered at the tertiary care center. We tested this hypothesis with the use of data obtained from our region. These observations are relevant in the planning and budgeting processes of perinatal facilities.

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Material and methods

Women and Infants Hospital of Rhode Island (WIHRI) is a private, university-affiliated hospital serving as the perinatal tertiary care facility for Rhode Island and southeastern Massachusetts. The hospital is the only tertiary care facility between Boston, Massachusetts (75 miles northeast), and New Haven, Connecticut (100 miles southwest).

There are eight primary care obstetric facilities in the state of Rhode Island in addition to WIHRI, each having between 300 and 1800 deliveries per year. WIHRI delivers between 40% and 60% of the state's 14,000 live births per year. In Southeast Massachusetts, there are

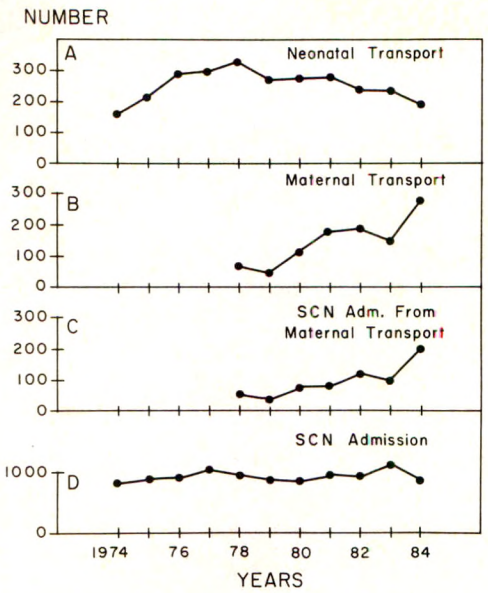


Fig. 1. *A*, Total number of neonatal transports per year since 1974. *B*, Total number of maternal transports per year since 1978. *C*, Total number of infants per year who were admitted to the special care nursery, who were born to mothers who were transported, since 1978. *D*, Total number of admissions per year to the special care nursery since 1974.

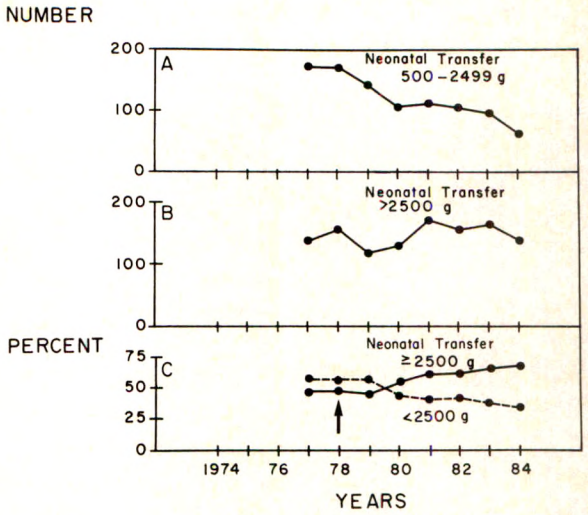


Fig. 2. *A*, Total number of neonatal transports per year, >500 and ≤2499 gm, since 1977. *B*, Total number of neonatal transports per year, ≥2500 gm, since 1977. *C*, Percentage of infants <2500 and ≥2500 gm who were neonatal transports per year since 1977. The *arrow* at the bottom denotes the year formal maternal transfer analysis began.

five obstetric facilities delivering a total of 6000 babies per year, none of which is a tertiary care center. Thus WIHRI is the only tertiary care center for the 13 hospitals within the region, with 20,000 live births per year.

WIHRI began its tertiary care center operation in 1974, with the establishment of a special care nursery, along with a neonatal transport team. In 1978, the maternal-fetal medicine program was added with strong emphasis on maternal transport in high-risk pregnancy. We define maternal transports as those pregnant women who originally elected to be delivered in hospitals within the region, but who, because of complications, were transported to WIHRI, usually by decision of the primary care obstetricians. No specific records were kept on the mode of transport. Some patients came by ambulance as would be common if the patient was in labor, and others by self-transport if labor was not imminent.

To evaluate the effects of an increasing number of maternal transports on the patterns of admission at a tertiary care facility, the following data were collected from annual hospital statistics: live births, neonatal and maternal transports, the number of special care nursery admissions of infants who were born to women who were transported antenatally, and total admissions to the special care nursery. The data were subdivided into two birth weight categories: >2500 and ≤2500 gm. For the maternal transports, the data are available only since 1978. There were some maternal transports before 1978 but the number was small, and complete data are not available.

Statistical analysis was by χ^2.

Results

From 1973 through 1984, there was an increase in live births at WIHRI, from 5300 in 1973 to 7317 in 1984. The number of low birth weight infants (≤2499 gm) born at WIHRI also increased. In 1973, 6.7% of the live births were low birth weight; by 1984, this had increased to 8.7% ($p < 0.001$ by χ^2 analysis).

Fig. 1 shows a gradual increase of infants transported to WIHRI during the period between 1974 and 1978 (154 in 1974 to 333 in 1978). From 1978 through 1984, there was a gradual decline from 333 to 198. During the same period of time, maternal transports increased from 65 in 1978 to 280 in 1984. Correspondingly the numbers of infants admitted to the special care nursery who were born to women transported to WIHRI increased from 43 in 1978 to 201 in 1984. During this time period the total number of admissions to the special care nursery remained stable at around 950 to 1000 per year.

Fig. 2 depicts the actual number of neonatal transports by birth weight: low birth weight (>500 and ≤2499 gm) and term (>2500 gm) infants. The number of low birth weight infants transported decreased over the years. In contrast, the number of term infants transported remained unchanged. Thus the percentage of term infants transported to our facility increased from 44.6 to 67.2 between 1977 and 1984 ($p < 0.001$).

Comment

The purpose of this report is to document how the patterns of modern perinatal care affect delivery of health care at a tertiary care facility. There are several reports of improvement in perinatal mortality and morbidity as a result of perinatal regionalization.[3-5] What has not been evaluated is the effect of regionalization on admission and utilization patterns in a tertiary care center.

The rise in the number of live births in the tertiary care center from 1974 to 1984 is partly due to an increase in the number of births of low birth weight infants. This is to be expected because of heightened awareness on the part of primary care physicians of the most beneficial location for the delivery of the high-risk infant. Furthermore, the establishment of the subspecialty of maternal-fetal medicine and the availability of perinatologists in the tertiary care center has also stimulated increased maternal transports. With the increased number of preterm pregnant women being transported to the tertiary care center, the percentage of low birth weight infants increased accordingly. The continuation of comparable numbers of term infants being transported to the tertiary care center reflected a phenomenon that was not unexpected: While the primary care physicians readily recognized the imminent high-risk potential of a preterm pregnancy (thus referring the mother to a tertiary care center), there was less accuracy in identification of the high-risk term pregnancy whose infant would require referral. The latter accounts for the increased percentage of term infants in the neonatal transport statistics.

From the administrative and fiscal perspective, it has become apparent that establishment of a maternal-fetal medicine program in a tertiary care center may require commitment of additional resources to this component when statistics indicate a trend toward more maternal transports. At the same time, allocation of fiscal resources to the neonatal program should remain unchanged despite a fall in the number of neonatal transports since the total number of nursery days would remain unchanged. Since total admissions to the special care nursery are constant, there is a need for allocation of resources within a community or region to the tertiary care center for delivery of the appropriate level of care.

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Septic shock with renal failure after chorionic villus sampling

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A report of septic shock and renal failure after a chorionic villus sampling is presented. Uterine evacuation, antibiotics, and vasopressor therapy were unsuccessful in reversing hypotension, coagulopathy, and anuria. The patient's condition improved only after exploratory laparotomy and removal of the uterus, ovaries, and fallopian tubes. The incidence of such serious complications must be determined. (Am J Obstet Gynecol 1986;154:1100-2.)

Key words: Chorionic villus sampling, septic shock, renal failure, coagulopathy

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Chorionic villus sampling has recently become available for the prenatal diagnosis of genetic disorders in the first trimester of pregnancy. The question of safety of the procedure has been raised, but no serious maternal complications have been reported. The following is a case report of a patient transferred to the Kaiser Foundation Hospital in San Francisco, California, for acute dialysis after chorionic villus sampling complicated by septic shock, disseminated intravascular coagulation, and anuria.

Case report

A 34-year-old, gravida 2, para 0 woman underwent chorionic villus sampling at 11½ weeks' gestation. Cultures for gonococci and chlamydia were both negative before the procedure. The procedure required two catheter insertions and was without incident. Four days later the patient presented to the emergency room complaining of fever and vaginal spotting. There was no history of self-instrumentation. Pelvic examination revealed a nontender uterus consistent with 10 to 12 weeks' gestation. Temperature was 37° C and the leukocyte count was 8900/mm³. The patient was diagnosed as having the "flu" and sent home with instructions to return if she developed higher temperatures. She returned 6 hours later with shaking chills and a temperature of 38.9° C. Pelvic examination again revealed a nontender uterus and no masses. The patient was admitted for intravenous hydration and observation.

At the time of admission, the leukocyte count had decreased to 3500/mm³ with 38% segmented cells and 57% band cells noted. Five hours after admission pelvic examination revealed marked uterine tenderness, and a diagnosis of septic abortion was made. She was begun on a regimen of intravenous cefoxitin, 2 gm every 6 hours, and gentamicin, 80 mg every 8 hours, and shortly thereafter a suction curettage was performed while she was under general anesthesia. After evacuation of the uterus the temperature rose to 40° C and blood pressure dropped to 60/30 torr. The leukocyte count decreased further to 1000/mm³ and clotting studies were consistent with a severe coagulopathy (platelets, 20,000/mm³; partial prothrombin time, >150 seconds; fibrinogen, <50 mg/100 ml). Clindamycin, 900 mg every 8 hours, was added to the antibiotic regimen. A dopamine hydrochloride infusion was begun and she was transferred to the intensive care unit.

Examination at this time revealed marked peripheral edema, bibasilar pulmonary rales, and abdominal tenderness and guarding. There was a small amount of bleeding from the vagina, the cervical os was closed, and the uterus was not palpable secondary to guarding. Ecchymosis and bleeding were noted from the venipuncture sites. A Swan-Ganz catheter was placed with a pulmonary wedge pressure of 24 torr. Urine output dropped to 28 ml for a 4-hour period with no response to increasing doses of furosemide. Creatinine levels increased progressively to 2.9 mg/100 ml. The patient received 20 units of platelets, two units of packed red blood cells, and four units of fresh-frozen plasma with no improvement in the clotting studies. She was then transferred to Kaiser Permanente Medical Center, San Francisco, California, for immediate dialysis.

Immediately after arrival a Scribner arteriovenous shunt was placed for hemodialysis. Gentamicin was discontinued because of worsening renal function, but cefoxitin and clindamycin were continued. The next day the patient was afebrile but there was no improvement of the disseminated intravascular coagulation. A dopamine infusion (20 μg/kg/min) was required to maintain a blood pressure of 90/60 torr. On pelvic examination the cervical os was noted to be closed, the lochia minimal and not foul, and there was no cervical motion tenderness. Diffuse abdominal tenderness and guarding continued. Ultrasound examination of the uterus was consistent with either residual blood clots or retained products of conception. Because there was no improvement in blood pressure or coagulation status, a repeat uterine evacuation was performed with removal of retained fetal skeletal parts.

The hemodynamic and coagulation status of the patient did not improve during the 20 hours after the repeat uterine evacuation. Because the tenuous status of the patient persisted, total abdominal hysterectomy and bilateral salpingo-oophorectomy were carried out with the patient under general anesthesia.

At the termination of the operation, vasopressor therapy was no longer necessary to maintain normal blood pressure. Clotting parameters improved after operation and were normal within 24 hours. The pulmonary function improved rapidly during the next 48 hours.

Blood cultures from the initial emergency room visit were reported positive for *Peptostreptococcus*, probable *Peptococcus*, and a gram-negative anaerobe. All subsequent cultures were negative. The patient was continued on a regimen of clindamycin for a total of 14 days. Anuria persisted after operation until the day before discharge, when she voided 150 ml of urine. She was discharged home on hospital day 19 to be followed up with outpatient hemodialysis three times weekly. Two weeks after discharge, dialysis was no longer required.

Pathologic examination of the uterus revealed endometritis, myometritis, and fibrin thrombi containing trophoblasts within the myometrium. No pathologic abnormalities were noted in the ovaries and tubes.

Comment

Chorionic villus sampling was recently introduced in this country as a technique for early prenatal diagnosis. Its popularity has increased rapidly. Because chorionic villi can be obtained directly by transcervical aspiration in the first trimester of pregnancy, fetal cells can be analyzed for chromosomal and biochemical defects immediately. Amniocentesis, by contrast, is not performed before the sixteenth week of gestation and results are not available for an additional 2 to 3 weeks until the fetal cells are grown in tissue culture and analyzed.

Thus chorionic villus sampling offers patients the psychological advantage of knowing if they have an affected offspring at an earlier stage of gestation. If a woman chooses to terminate her pregnancy, a first-trimester abortion is safer, less costly, and more acceptable psychologically.

Although an attractive alternative, chorionic villus sampling has potential risks. Infection, rupture of the fetal membranes, spotting, frank bleeding from the sampling site, dissection of the chorion frondosum, and a rate of fetal loss greater than that seen with amniocentesis have all been reported.[1] A careful search of the literature, however, failed to reveal any reports of serious maternal complications, including septic shock.

Spotting appears to be a common complication, usually not associated with long-term sequelae.[1] Our patient presented with spotting and fever; she had no abdominal pain or pelvic tenderness. When it became apparent that infection was present, antibiotics were started and uterine evacuation was carried out. After a second dilatation and evacuation procedure yielded retained fetal parts, there was hope that she would begin to improve because an obvious nidus for infection had been removed. Conservative management of septic shock and renal failure in patients with septic abortions has been advocated by Hawkins et al.[2] in order to preserve fertility. Our patient, although stable on a regimen of therapeutic doses of antibiotics, pressor therapy, and hemodialysis, did not improve during 20 hours after a second uterine evacuation; it became necessary to proceed with an exploratory laparotomy, a total hysterectomy, and a bilateral salpingo-oophorectomy.

The seriousness of this complication after chorionic villus sampling raises the question of safety of the procedure. Clearly the frequency of serious complications must be determined. The unfortunate outcome in this case has led us to propose the following recommendations: (1) continued presampling screening and treatment of existing infection; (2) use of strict aseptic technique; (3) limitation of the number of catheter insertions and use of a new catheter for each sampling attempt; (4) inclusion in the consent form under the statement of risks, "possible serious infection requiring surgical intervention"; (5) counseling of all patients to abstain from sexual intercourse for a period of several days after the procedure.

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Relationship of baseline fetal heart rate to gestational age and fetal sex

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A study of 37 patients who underwent 365 antepartum fetal heart rate tests showed a significant difference in heart rate between 19 to 24 weeks' and 36 to 40 weeks' gestation. Baseline heart rate remained within the normal range, suggesting that an abnormal heart rate at any gestational age should prompt further fetal assessment. Baseline fetal heart rate was not significantly different between male and female fetuses.
(Am J Obstet Gynecol 1986;154:1102-3.)

Key words: Baseline fetal heart rate, gestational age, fetal sex

Baseline fetal heart rate has long been used as an indication of fetal status. The currently acceptable range of a normal baseline fetal heart rate is 120 to 160 bpm, with >160 bpm designated as "tachycardia" and <120 bpm as "bradycardia."[1, 2]

The most common cause of baseline tachycardia is maternal-fetal fever, with chronic hypoxemia as the more ominous cause in other instances. Fetal tachyarrhythmias and maternal thyrotoxicosis are rare causes of tachycardia. Prematurity, with apparent "immaturity" of the autonomic nervous system, has long been

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Table I. Mean baseline fetal heart rate

Fetuses	*Estimated gestational age*				
	19-24 wk	*24-28 wk*	*28-32 wk*	*32-36 wk*	*36-40 wk*
Male	143 ± 8	143 ± 6	136 ± 8	134 ± 14	131 ± 8
Female	144 ± 4	143 ± 6	139 ± 6	138 ± 6	133 ± 8
Total	143 ± 7*	143 ± 6	137 ± 7	136 ± 7	132 ± 8*

*$p < 0.01$.

thought to be related to physiologic tachycardia. Causes of fetal bradycardia include acute hypoxia, congenital heart lesions, and congenital heart block.[1, 2] There is also a popularly held view that baseline fetal heart rate may be an indicator of fetal sex.

This study was undertaken to determine the antepartum baseline fetal heart rate in a group of patients who subsequently had normal pregnancy outcomes.

Material and methods

Thirty-seven patients underwent 365 antepartum fetal heart rate tests at 19 to 40 weeks' gestation. Indications for testing included prematurity in six cases, gestational diabetes in five, collagen vascular disease in three, and miscellaneous in seven. Eleven low-risk patients volunteered as controls for the study. Baseline fetal heart rate was defined as the average baseline fetal heart rate in a 10-minute period, rounded off to the nearest 5 bpm. All neonates were appropriate for gestational age; nine were delivered at ≤36 weeks. Five-minute Apgar scores were all ≥8. There were no congenital anomalies. There were 25 male and 12 female singleton births. None of the patients had thyrotoxicosis, and no fetal arrhythmias were detected.

Results

There was a statistically significant difference in baseline fetal heart rate between 19 to 24 weeks' and 36 to 40 weeks' gestation; there was no difference in baseline fetal heart rate between male and female fetuses at any gestational age (Table I).

Comment

While the difference in baseline fetal heart rate between 19 to 24 weeks' and 36 to 40 weeks' gestation was statistically significant, baseline fetal heart rate was never outside the "normal range" of 120 to 160 bpm. Maturation of the autonomic nervous system may account for the difference between the early and the late gestational age groups. The presence of abnormal fetal heart rate at any gestational age merits further investigation. Fetal sex has no influence on baseline fetal heart rate.

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Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon

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A vertical radiolucent fissure is frequently seen in the symphysis pubis on x-ray pelvimetry studies. This phenomenon is illustrated and discussed. It appears to be a parameter of targeted relaxin softening and stretching of the symphysis. (Am J Obstet Gynecol 1986;154:1104-5.)

Key words: Relaxin, pubic symphysis gas phenomenon, pregnancy

Relaxin activity during pregnancy is associated with loosening, widening, and increased flexibility of the symphysis pubis. Late in pregnancy or during parturition, associated with such softening is a vertical fissure within the symphysis, which is frequently seen on the x-ray film as a dark streak of gas (Fig. 1).

Previous radiologic studies of this phenomenon, now contraindicated, confirm this targeted hormonal response of the symphysis to pregnancy. I searched for this finding in 100 unselected x-ray pelvimetry films obtained late in pregnancy.[1] All of the cases were of cephalic presentations. With the use of strict criteria and after the elimination of doubtful and even probably positive films, this finding was evident in 30 of the 100 cases. To test whether cephalic presentation affected the results, 22 unselected breech presentations were studied. Six of these (31.3%) were positive, the similarity of results suggesting a hormonal relationship. Parity, pelvic size, and pelvic shape did not play a significant role. Pain was infrequent. Williams[2] studied the x-ray pelvimetry results of 116 primigravid women and 116 multigravid women. The streak was detected in 41.5% of the patients, with no essential difference between the two groups. Age played no part. While the phenomenon was most common in late pregnancy or during parturition, on occasion, it was noted during the first or second trimester. In most instances the gas shadow disappeared within a week after delivery. In neither study was the outcome of labor deleteriously affected by the phenomenon.[1, 2]

Normally the symphysis is resistant to separation. It contains a compressed central fissure, unlined by synovial membrane, which is not visible on an x-ray film.

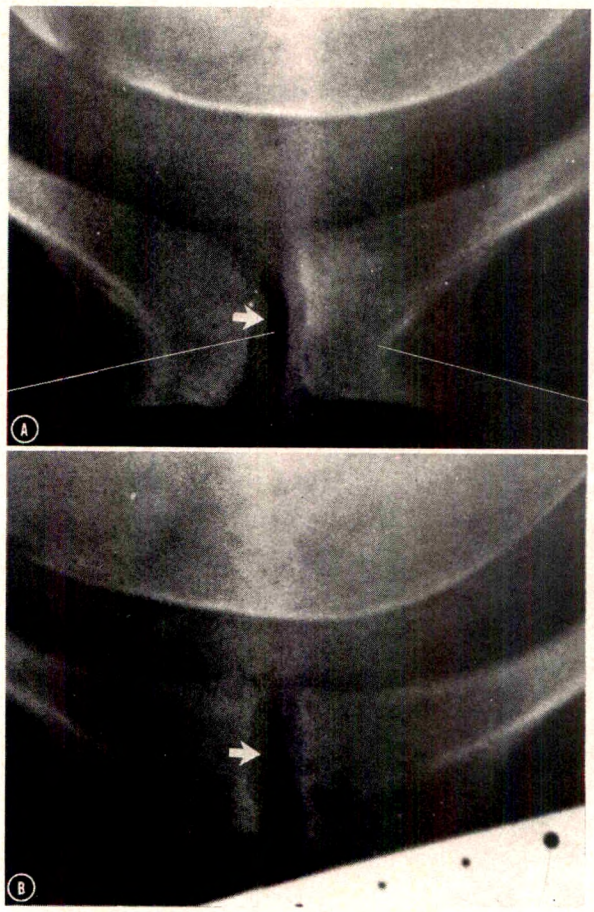


Fig. 1. *A* and *B*, Two examples of gas in the pubic symphysis during pregnancy.

This potential space may become real under the changes described with pregnancy. A negative pressure balance is created, followed by transmission of gas to fill the vacuum. Rarely, the visible cleft has been described with trauma and degenerative disease, but essentially it is a phenomenon of pregnancy. Occasionally the clefts are multiple or associated with tiny avulsions from the contiguous cortex, suggesting small components of

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trauma and/or multiple anatomic clefts. Williams[2] found that with successive pregnancies the shadows tended to be the same, favoring an anatomic basis.

Relaxin has been detected in the nonpregnant state and even in men, but its greatest concentrations normally arise from the corpus luteum of pregnancy. It changes the biochemical nature of the cartilagenous connective tissue of the symphysis, resulting in its softening and increased flexibility. The radiologic visibility of the fissure is a parameter of such softening and stretching. When detected, it had no known harmful effect on the outcome of delivery. Indeed, as a component of pubic laxity, it probably reflects a useful concomitant of pregnancy. Its full implications will not be understood until the precise role of relaxin and its complex relationships to the other hormones of pregnancy are elucidated. Although this phenomenon has been described previously, this is the first report relating it to relaxin.

Assistance given by Ms. Mary Doherty, research librarian, and Philip Mullan, Department of Medical Illustration, is acknowledged with thanks.

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Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization

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The successful use of ultrasound-guided fetal umbilical venipuncture and direct intravascular transfusion in a case of severe hydrops fetalis at 28 weeks' gestational age is described. Complete reversal of the hydropic changes was observed in utero. The use of this new technique in fetal treatment of Rh disease is discussed. (Am J Obstet Gynecol 1986;154:1105-7.)

Key words: Hydrops fetalis, ultrasound, transfusion, antenatal

Direct intravascular fetal blood transfusion in the fetus severely affected by Rh immunization has been reported with the use of fetoscopy,[1] ultrasonically guided umbilical venipuncture,[2] and guided cannulation of the umbilical vein within the fetal liver.[3]

Reported here is a case of severe hydrops fetalis at 28 weeks' gestation treated with a combination of intravascular and intraperitoneal transfusions. Complete reversal of the hydropic condition in utero was observed.

Case report

The patient, a 32-year-old woman, gravida 2, para 2-0-0-2, was referred to North Carolina Memorial Hospital at 28 weeks' gestation because of Rh immunization (anti-D). Sonography before referral showed severe hydrops fetalis. Amniocentesis was performed and the amniotic fluid optical density shift at 450 nm was reported to be 0.495.

Repeat ultrasound examination on arrival confirmed the presence of massive fetal ascites (Fig. 1), and moderate skin edema. No pleural or pericardial effusions were present. The placental thickness was 8.2 cm. Because of the severity of the condition, ultrasound-guided venipuncture and direct transfusion were undertaken.

The umbilical insertion on the placenta was identified, and a likely venipuncture site was chosen (Fig. 2). With the patient under parenteral sedation and local anesthesia, a 19-gauge needle catheter was placed percutaneously into the amniotic cavity, and the needle trocar was removed. A 21-gauge transfusion needle 18 cm long with a 26-gauge tip[1] was inserted through the cannula into the amniotic cavity and advanced under ultrasonic guidance to the umbilical vein. The umbilical vein was punctured and fetal blood aspirated. The fetal hematocrit was 14%, the fetal Po_2 was 20 mm Hg, and the Pco_2 was 45 mm Hg. An isovolumetric exchange was begun in 5 ml increments with the use of 90%

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Fig. 1. This transverse midabdominal view of the fetus demonstrates the lower liver mass surrounded by ascitic fluid *(black area)*.

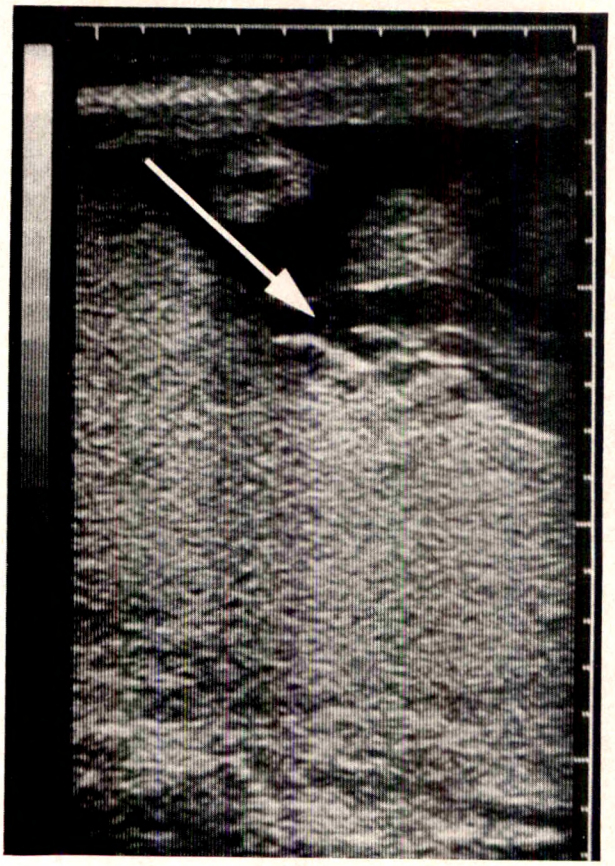


Fig. 2. This transverse sonogram of the upper right uterine fundus demonstrates the posterior placenta with the umbilical vein insertion. The *arrow* indicates the umbilical vein on the surface of the placenta and duplicates the approach used for umbilical venipuncture under ultrasound guidance.

packed, washed, irradiated, type 0, Rh-negative red blood cells to a total of 29 ml, after which aspiration was no longer possible. An additional 55 ml of cells was then slowly infused. No bleeding was apparent after needle removal. A 16-gauge Touhy needle was then inserted into the fetal peritoneal cavity and 200 ml of clear, yellow fetal ascitic fluid was removed. Eighty milliliters of donor red blood cells was infused into the peritoneal cavity. The fetal heart rate remained stable throughout these procedures.

One week after the transfusion, placental thickness was 7.1 cm. Two weeks later, fetal skin edema was gone, and by 3 weeks after transfusion there was no free fetal peritoneal fluid visible on ultrasound. One additional intraperitoneal transfusion of 110 ml of 90% packed cells was performed at 31½ weeks' gestation without complication.

Ultrasound at 34½ weeks showed no signs of hydrops. Amniocentesis was performed and the lecithin-sphingomyelin ratio was found to be 2.2. Delivery was by repeat cesarean section at 35 weeks, and the 2480 gm female infant had Apgar scores of 8 and 9 at 1 and 5 minutes. There were no signs of hydrops. The central neonatal hematocrit was 42% and only two fetal cells per 10,000 were seen with Kleihauer-Betke testing of cord blood. The neonate experienced mild transient tachypnea but required no exchange transfusion and was discharged with the mother.

Comment

Rodeck et al.[1] documented the potential utility of intravascular fetal transfusions by means of fetoscopy in early pregnancy. Fetoscopy was not considered here as it requires the intrauterine intrusion of a larger instrument, and intra-amniotic visibility is severely limited at 28 weeks' gestation by turbidity.

Ch. de Crespigny et al.[3] recently reported 16 direct transfusions in four cases with the use of a 20-gauge needle placed into the umbilical vein within the liver through an 18-gauge needle that had been inserted into the fetal liver substance itself. Reversal in utero of mild hydrops with survival did occur in one case with their technique. They reported no fetal complications. Ultrasound-guided umbilical venipuncture was used to sample fetal blood in 13 cases and to transfuse one hydropic fetus in a report from Hobbins et al.,[2] but the details of the hydropic case and subsequent outcome were not given. They used a 25-gauge needle and emphasized transplacental placement with transplanar (scan plane across axis of needle) needle imaging with a linear array transducer.[2] Here, the technique of Hobbins et al. was modified by use of a needle with a larger, stiffer shaft that allowed for relatively easy transamniotic placement with coplanar (axis of needle parallel to scan plane) ultrasound guidance. The transcutane-

ous-transmural catheter sleeve allowed easy movement of the transfusion needle and no fluid leakage occurred.

Transamniotic ultrasound-guided fetal umbilical venipuncture offers direct access to the fetal circulation for sampling as well as infusion and may offer the severely hydropic fetus a greater probability of survival. This technique might subject the fetus to less organ trauma than the transhepatic approach advocated by Ch. de Crespigny et al.[3] It is premature to propose the replacement of the more traditional techniques of intraperitoneal transfusion in the care of the less severely affected fetus until more experience is acquired, but the intravascular approach may prove superior.

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Factors influencing nonstress test results in multiple gestations

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The nonstress test remains the most widely used method of antepartum fetal health assessment in multiple gestations. However, few data are available on factors influencing its results in those cases. Our observation on 530 such tests has shown results similar to those in singleton gestations. Prematurity was associated with a higher rate of nonreactive tests; however, order and mode of presentation had no impact. (Am J Obstet Gynecol 1986;154:1107-8.)

Key words: Twins, multiple gestation, nonstress test, antepartum testing

Among methods of antepartum fetal health assessment in use today the nonstress test (NST) appears to be the most widely used. The reactivity of the fetal heart rate (FHR) has been shown to be a function of gestational age in singleton fetuses.[1] Other factors that may influence results are less well known or characterized. In multiple gestations, antepartum fetal health assessment also relies mainly on the NST. Therefore we designed this study to determine which factors in multiple gestations might influence test results.

Material and methods

Our study was both retrospective and prospective: the retrospective part between January 1, 1980, and June 30, 1983, and the prospective part between July 1, 1983, and March 12, 1984. During that period of time, at our institution, we delivered 61,932 infants, including 610 multiple gestations (0.98%). Ninety sets of multiple gestations (183 fetuses) had at least one NST performed. Fifty-one sets in the retrospective period (one triplet gestation) had 218 NSTs and 39 sets in the prospective (two triplet gestations) had 312 NSTs. Two fetuses died in the antepartum period before the onset of antepartum testing and were thus excluded from the study. Thus 530 individual NSTs in fetuses that were alive at the onset of testing were available for analysis. All NSTs were performed simultaneously on each fetus of a multiple set, the patient being in a semi-Fowler position. A reactive NST was defined as the occurrence of two FHR accelerations of 15 bpm and 15 seconds' duration in a 10-minute window. All other results were considered nonreactive. Statistical analysis of discrete data was performed with χ^2 test with Yates' correction when appropriate. Significance was set at a p value <0.05.

Results

Table I shows the distribution of NST results by gestational age. Globally, 86.6% (459 of 530) of the tests were reactive. Fetuses between 26 and 31 weeks' gestation had a reactive NST in only 68.1% of cases (49 of 72), while those between 32 and 36 weeks had a

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Table I. Distribution of NST results by gestational age in twins

Gestational age (wk)	Reactive		Nonreactive		Total
	n	%	n	%	
26	0	0	2	100	2
27	0	0	2	100	2
28	11	73.3	4	26.7	15
29	8	66.7	4	33.3	12
30	11	68.8	5	31.2	16
31	19	76.0	6	24.0	25
32	36	78.3	10	21.7	46
33	46	82.1	10	17.9	56
34	61	88.4	8	11.6	69
35	63	88.7	8	11.3	71
36	84	95.5	4	4.5	88
37	43	91.5	4	8.5	47
38	32	100	0	0	32
39	23	92.0	2	8.0	25
40	11	91.7	1	8.3	12
41	9	90.0	1	10.0	10
42	2	100	0	0	2
Total	459	(86.6)	71	(13.4)	530

reactive NST in 87.9% of cases (290 of 330), $p < 0.001$, and those between 37 and 42 weeks in 93.8% of cases (120 of 128), $p < 0.001$. The difference between these last two groups was not significant.

Order of fetuses at the time of testing had no influence on results as reactive NSTs were observed in 85.8% of first babies (224 of 261) and 87.4% of second babies (235 of 269).

Finally fetuses in breech presentation were more likely to have a nonreactive NST (29 of 164, 17.7%) than those in vertex or transverse-oblique presentations (42 of 366, 11.5%). However, this difference was not statistically significant.

Comment

The NST remains the most widely used method of antepartum fetal assessment. Previous studies have demonstrated that the frequency of FHR accelerations is lower in premature fetuses. This high nonreactivity rate is considered to be related to the immaturity of the central nervous system and/or myocardium and to a higher baseline heart rate; the heart often cannot accelerate sufficiently to meet the accepted criteria of reactivity.[1] Our study in multiple gestations confirms this previous observation made in singleton gestations with a constant progression in the rate of reactivity between 26 weeks and term. The overall rate of reactivity in our multiple gestations is comparable to what has been observed in singleton pregnancies with 86.6% of tests being reactive.[2] Furthermore there was no difference in the rate of reactivity depending on the order of fetuses at the time of testing. However, we had no way of knowing if that order remained the same during all tests performed on each fetus. Thus we cannot conclude that this relation remained the same as that at delivery. Nonetheless it is noteworthy that the second fetus (at the time of testing) had a similar rate of reactivity, in comparison with that of the first fetus, as many studies have shown higher perinatal morbidity and mortality rates for the second twin. On careful review of these data, antepartum complications do not seem to be a significant factor with regard to this fact but rather it seems that intrapartum events must play a more important role.

Finally, fetal presentation had no significant influence on the rate of reactivity in multiple gestations. A recent report by White and Cibils[3] has shown a lower incidence of intrapartum FHR accelerations in breech presentations compared with that expected with a vertex presentation, but these data do not support this conclusion in the antepartum period.

Our data show that special care must be taken in the interpretation of a nonreactive NST in multiple gestations, especially at a shorter gestational age. The likelihood of a falsely nonreactive NST in those fetuses <32 weeks' gestation is significant, as 31.9% of fetuses had a nonreactive NST in that gestational age group. Logically, one does not want to compromise a healthy fetus by delivery in multiple gestations simply because one of them is nonreactive. This is particularly true in the premature age group. Therefore there is a definite need to carefully evaluate other means of assessing the fetus when one or more tests of fetal well-being suggest compromise.

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Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death

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An unexplained intrauterine death occurred within 12 hours of a normal reactive nonstress test. Furthermore the umbilical arterial impedance measured by pulsed Doppler ultrasound was also normal.
(AM J OBSTET GYNECOL 1986;154:1109-10.)

Key words: Nonstress test, Doppler ultrasound, blood flow, umbilical artery, intrauterine death

The nonstress test is now widely regarded as being one of the best methods of assessing fetal well-being particularly in the management of the postterm fetus. Nevertheless intrauterine deaths have occurred after a normal tracing. Most of these were due to acute events but a small number remain unexplained.

Pulsed Doppler ultrasound permits both quantitative and qualitative calculation of blood flow velocity within fetal vessels, particularly the umbilical artery, and fetuses with a high impedance within this vessel have been found to be seriously compromised.[1]

A case is presented in which a fetus having both a normal reactive heart rate tracing and a normal umbilical arterial impedance died in utero from unexplained causes some 12 hours later.

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Case presentation

A 38-year-old primigravid woman first presented at 13 weeks of amenorrhea and gestation was confirmed 3 weeks later by ultrasound.

The antenatal course was uneventful until no fetal heart movement was detected at term plus 11 days. A nonstress test the previous day was reactive (Fig. 1). On ultrasound scanning at the same time there was no oligohydramnios and the placental architecture was Grade I. The umbilical arterial impedance measured by pulsed Doppler ultrasound was also within the normal range (Fig. 2) previously established with the same technique.[1] Intrauterine death was confirmed on ultrasound the next morning. Delivery was effected within 24 hours and the fetus was female, was grossly normal apart from some skin maceration, and weighed 3360 gm, with no evidence of cord entanglement or torsion. There was no retroplacental clot.

Kleihauer-Betke test immediately after birth detected no fetomaternal bleeding. Glycosylated hemoglobin (hemoglobin A_{1c}) was 0.052 (normal range = 0.03 to 0.06). Results of a TORCH screen (toxoplasma, rubella virus, cytomegalovirus, and herpesvirus) were

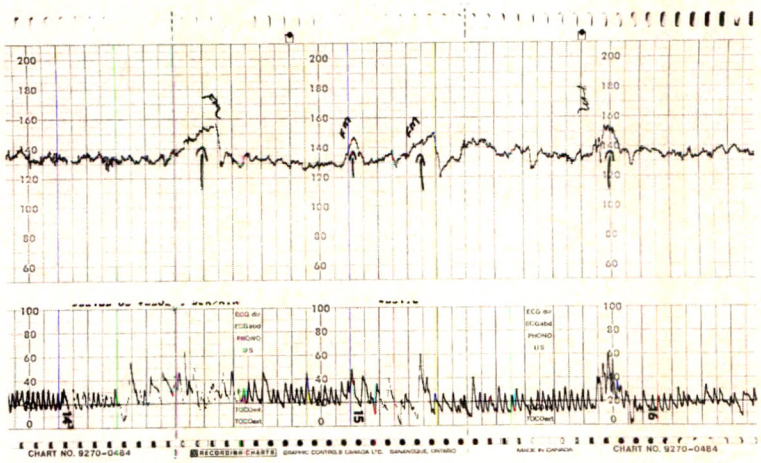


Fig. 1. Typical section of the normal fetal nonstress test recorded less than 12 hours before intrauterine death.

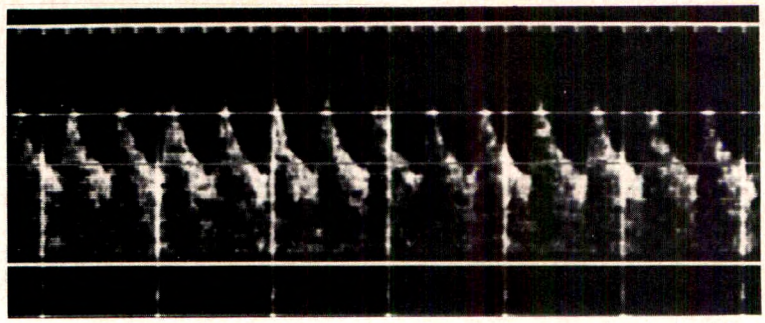


Fig. 2. Normal umbilical arterial sonograms obtained from the fetus less than 12 hours before intrauterine death. The pulsatility index (peak-to-peak height/mean height) was 0.57 (upper limit of normal = 0.85).

unchanged from those of a similar screen performed at 13 weeks' amenorrhea. There was no change in maternal hemoglobin concentration during the 24-hour period in which fetal death occurred. Maternal blood group was O positive but with a positive direct Coombs' test, indicating that the red blood cells were coated with complement, although this was subsequently shown to be due to cold agglutinins.

The placenta weighed 600 gm and histologic examination detected only one small area (1 by 1 by 1 cm) of infarction near the periphery. The cord, measuring 32 cm in length, contained three blood vessels with no evidence of thrombosis or inflammation.

Postmortem examination of the fetus revealed no gross anatomic or histologic abnormality except extensive skin and brain maceration.

Comment

Intrauterine death after a normal reactive nonstress test has been reported, but the vast majority of such deaths have been due to acute accidents of the cord, infection, fetal anomalies, or abruptio placentae. The unexplained death of a normal fetus within 48 hours of a normal reactive nonstress test has only been reported twice, once within 24 hours of a normal tracing and the other immediately following a normal reactive tracing. The case presented is the first such death to be documented within a short period of a normal umbilical arterial impedance measurement. We view this as a cautionary warning to those groups examining this technique as a research procedure and particularly to those advocating it as a reliable and sensitive measure of fetal well-being.[2]

We thank Dr. M. Barkin for permission to report his case.

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The in vivo effect of amniotic fluid on endocervical microflora

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To test the in vivo effect of amniotic fluid on endocervical microflora, we performed endocervical cultures before and after rupture of the membranes in 20 patients in term labor. There were no significant qualitative or quantitative differences in aerobic or anaerobic isolates for up to 4 hours after membrane rupture. Group B streptococci, isolated in two patients (10%), persisted after rupture of the membranes. These data suggest that endocervical cultures are minimally influenced by rupture of the chorioamnion for at least 4 hours. (Am J Obstet Gynecol 1986;154:1111-2.)

Key words: Amniotic fluid, bacterial inhibition

Endocervical cultures are commonly recommended in patients with spontaneous rupture of the membranes before labor.[1] Although amniotic fluid is known to have bacterial inhibitory activity in vitro,[2] the effect of amniotic fluid on endocervical cultures is not established. The purpose of this study was to evaluate the in vivo effect of amniotic fluid on the endocervical microflora.

At Medical Center Hospital in San Antonio, Texas, patients presenting in uncomplicated labor at term were evaluated as possible candidates for study. Inclusion criteria were an intact chorioamnion and no digital examination with bactericidal or bacteriostatic agents. Patients later found to have meconium-stained amniotic fluid were excluded.

Consenting patients underwent sterile speculum examination. The cervix was visualized and, with the use of prereduced swabs, endocervical samples were plated directly onto appropriate media for aerobes, anaerobes, and genital mycoplasmas. The chorioamnion was then ruptured according to standard obstetric indications, and before repeat digital examination, sterile speculum examination and culture were repeated.

Bacterial and mycoplasma isolates were identified by use of standard techniques. The plate streak methodology was used for bacterial semiquantitation.

To study the effect of increasing duration of ruptured membranes on culture results, the patients were grouped according to time interval of rupture of the membranes between cultures. Group 1 included patients with rupture of the membranes <2 hours between cultures, and group 2 comprised patients with rupture of the membranes ≥2 hours between cultures. Longer durations of ruptured membranes were not possible because of the need to examine patients in labor. With each patient used as her own control, we evaluated numbers of bacterial isolates before and after rupture of the membranes by paired Student's *t* test. A value of $p \leq 0.05$ was considered significant.

Table I. Bacterial isolates in 20 patients in labor at term before and after rupture of the membranes

Isolates	Patients with positive culture			
	Before rupture of membranes		After rupture of membranes	
	n	%	*n*	%
Lactobacillus sp.	18	90	19	95
Staphylococcus epidermidis	14	70	16	80
Ureaplasma urealyticum	12	60	13	65
Bacteroides bivius	8	40	8	40
Candida sp.	4	20	3	15
Mycoplasma hominis	3	15	2	10
Bacteroides fragilis	2	10	3	15
Anaerobic *Streptococcus* sp.	1	5	2	10
Group B streptococci	2	10	2	10
Enterococci	0	0	2	10
Clostridium sp.	2	10	2	10

Twenty patients were admitted into the study. Groups 1 and 2 contained 10 patients each. Mean age and parity were 24 years and 1.6, respectively. Mean gestational age was 41 weeks. Age, parity, and gestational age were similar between groups 1 and 2.

In group 1, cervical dilatation was 5.3 ± 1.5 cm at admission and 6.8 ± 2.1 cm at the time of repeat culture, whereas in group 2, cervical dilatation was 3.3 ± 0.63 cm initially and 5.6 ± 2.6 cm at the time of

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repeat culture. There was no significant increase in cervical dilatation in either group.

Isolation rates of organisms are shown in Table I. Group B streptococci were isolated in two patients. There were no significant changes after ruptured membranes.

In groups 1 and 2, the duration of rupture of the membranes was 0.72 ± 0.36 and 3.72 ± 0.70 hours, respectively. In group 1, the mean number of aerobic bacterial isolates was 2.0 ± 0.47 before rupture of the membranes and 2.1 ± 0.57 after rupture of the membranes. There were 1.2 ± 1.0 anaerobic isolates before rupture of the membranes and 1.1 ± 1.3 anaerobic isolates after rupture of the membranes. The numbers of aerobic and anaerobic isolates did not change significantly after rupture of the membranes. In group 2 there were 1.8 ± 0.42 aerobes before and 2.2 ± 0.79 aerobes after rupture of the membranes. There were 0.8 ± 0.9 and 1.3 ± 0.95 anaerobic isolates before and after rupture of the membranes. In group 2 there were no significant differences in numbers of aerobic and anaerobic isolates after rupture of the membranes. Group B streptococci, present in two of 20 patients, persisted after rupture of the membranes. There were no effects of rupture of the membranes on isolation rates of genital mycoplasmas.

Semiquantitation of the 77 bacterial isolates revealed that 25 (33%) increased in number, 19 (25%) decreased in number, and 35 (43%) remained unchanged after rupture of the membranes. Aerobes and anaerobes behaved similarly with respect to semiquantitation.

Endocervical cultures are commonly performed in patients with preterm spontaneous rupture of the membranes.[1] Research protocols collect data on endocervical infection as a pathogenetic mechanism in premature labor or premature membrane rupture, and clinical protocols attempt to identify carriers of group B streptococci of *Neisseria gonorrhoeae*. Research and clinical decisions depend on the reliability of such cultures. The in vitro bacterial inhibitory activity of amniotic fluid is well described. Although cross-sectional studies have demonstrated more anaerobic isolates among patients with preterm ruptured membranes, possible in vivo effects of amniotic fluid on culture results were not considered. In the present longitudinal study, endocervical bacterial isolates did not change qualitatively or semiquantitatively after ruptured fetal membranes. It is important that group B streptococci, present in 10% of patients, persisted in the presence of amniotic fluid. These data suggest that endocervical cultures are minimally influenced for at least 4 hours after rupture of the chorioamnion.

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Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section

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Single-dose trimethoprim-sulfamethoxazole prophylaxis after clamping of the umbilical cord decreased the rate of endomyometritis in high-risk patients by 50% when compared with placebo. This drug combination may affect the normal flora of the cervix the least so that superinfections should be rare. (AM J OBSTET GYNECOL 1986;154:1113-4.)

Key words: Prophylaxis, cesarean section delivery, endomyometritis

We evaluated the efficacy of a single dose of trimethoprim-sulfamethoxazole prophylaxis for cesarean section in high-risk patients (less than age 21). We chose this group of patients since data from our previous studies have indicated that patients less than 21 years of age are at a higher risk for developing postoperative infections than other groups.

Material and methods

The study included 57 patients and was conducted from November, 1983, to December, 1984. All patients who were less than 21 years of age and undergoing cesarean delivery at the University Hospital of the New Jersey Medical School in Newark, New Jersey, were considered candidates for the study. Informed consent was obtained from all participants. Candidates were excluded from the study if there was evidence of any of the following: treatment with an antibiotic within 2 weeks of delivery, any active infection and/or elevated temperature at the time of delivery, penicillin allergy, sulfa allergy, or internal fetal monitoring with either a scalp electrode or a uterine pressure probe to eliminate a possible cause of infection.

Patients were randomly divided into two groups. Group 1 received placebo and consisted of 28 patients; group 2 (29 patients) were given 240 mg of trimethoprim and 1200 mg of sulfamethoxazole in one dose intravenously after clamping of the cord at the time of delivery.

The patients were observed postoperatively for signs and symptoms of infection. A diagnosis of endomyometritis was determined by a temperature elevation ≥100.4° F on any two occasions during any 24-hour period from the second postpartum day until discharge from the hospital. A complete physical examination was performed to localize the site of infection, and complete blood count with differential count and urine, endocervical, and blood specimens for culture were obtained. Diagnosis of post–cesarean section endomyometritis was made on the basis of an elevated temperature, uterine tenderness, and the absence of another focus of infection. The majority of cases of infection had foul-smelling lochia. However, this was not considered necessary to make the diagnosis. Urinary tract infection was defined as fever ≥100.4° F and a positive urine culture of 10^5 organisms per milliliter or more. Wound infection was defined as fever ≥100.4° F and an abnormal-appearing wound with cellulitis or a wound draining purulent material. The treatment of endomyometritis included clindamycin and gentamicin or ticarcillin and clavulanic acid in appropriate doses. Statistical analysis of the data was performed by computer with the use of Fisher's exact test.

Table I. Comparison of groups

	Placebo	*Trimethoprim-sulfamethoxazole prophylaxis*
Number	28	29
Age (yr) (mean)	17.3	16.9
Gravidity	1.4	1.7
Vaginal examinations	3.9	4.1
Duration of labor (hr)	9.6	8.0
Duration of rupture of membranes (hr)	4.5	2.1
Elective repeat cesarean section	12	11

Results

The study groups were similar with regard to their demographic and obstetric parameters (Table I). All

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patients were of the lower socioeconomic class and more than 90% were black. There was no statistically significant difference between the two groups for any of the variables studied.

Of the studied population, 13 of 28 (46%) who received placebo developed endomyometritis after delivery compared with six of 29 (21%) who received single-dose prophylaxis with trimethoprim-sulfamethoxazole ($p = 0.036$ Fisher's exact test). The incidence of urinary tract infection and wound infection was similar.

The organisms most frequently isolated from patients who developed endomyometritis were peptococci, peptostreptococci, and *Escherichia coli.*

Comment

The results from this study are similar to those of previous studies that we have performed, indicating that prophylactic antibiotics for patients who were delivered by cesarean section decreased infection rates by approximately 50%.[1] Patients less than 21 years of age per se have been shown to have a higher rate of infection from our previous studies.

The high rate of endomyometritis in the placebo group may be related in part to our patients' lower socioeconomic status. Cunningham et al.[2] reported a similar incidence of endomyometritis in a similar population.

The results with trimethoprim-sulfamethoxazole administration are interesting since this combination would not be expected to reduce the anaerobic bacteria flora. Nevertheless, the combination did reduce infection by about 50%. These findings suggest several possibilities: that aerobes may be an important cause of post–cesarean section endomyometritis, that they are an active component of a mixed infection, or that the prophylactic regimen may have had some effect against the anaerobic flora. It is also possible that reduction in the aerobic flora permits postcellular defenses to cope more successfully with anaerobic invaders.

A single dose of the combination of trimethoprim-sulfamethoxazole deserves additional studies as a prophylactic agent. Also a single dose of antibiotic should save costs and be associated with fewer adverse reactions when compared with multiple dose regimens of antibiotic prophylaxis.

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Hydrops fetalis and chromosomal trisomies

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Three patients with trisomy 21 and one with trisomy 13 associated with nonimmunologic hydrops fetalis are presented. Analysis of previous nonimmunologic hydrops fetalis, which included chromosomal trisomies, and the present study yields an overall incidence of 7% for chromosomal trisomy disorders in live-born infants with nonimmunologic hydrops fetalis. Our data emphasize the need for chromosomal analysis of neonates with nonimmunologic hydrops fetalis. (Am J Obstet Gynecol 1986;154:1114-5.)

Key words: Hydrops fetalis, chromosome, trisomy

Hydrops fetalis can be divided into immunologic and nonimmunologic categories. As Rh disease has markedly diminished, nonimmunologic hydrops fetalis has become the most common type. Fourteen infants with nonimmunologic hydrops fetalis were managed at the University of Minnesota Hospitals from 1978 to 1983. Four patients (29%) had chromosomal trisomies (trisomy 21, n = 3; trisomy 13, n = 1).

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Patients

Maternal-fetal and neonatal characteristics are outlined in Table I. Ultrasonography revealed prenatal

Table I. Clinical data

Patient No.	*Pregnancy complications*	*Birth weight (gm)*	*Gestational age (wk)*	*Phenotypic characteristics*	*Genotypic diagnosis*	*Outcome*
1	Polyhydramnios; fetal scalp, thoracic edema	3040	35	Facial and truncal edema; ascites; features of Down's syndrome after diuresis	47,XY + 21 (skin fibroblasts)	Alive
2	Normal ultrasound at 20, 27 wk; polyhydramnios; fetal edema, ascites just before delivery	3840	35	Truncal edema, ascites	47,XY + 21 (skin fibroblasts)	Death, day 2
3	Premature labor	2540	32	Anasarca; features of Down's syndrome after diuresis; endocardial cushion defect with cor triatriatum (autopsy)	47,XY + 21 (bone marrow chromosomes)	Death, day 20
4	Severe preeclampsia with congestive heart failure	2460	34	Anasarca; multiple anomalies consistent with trisomy 13	47,XX + 13 (skin fibroblasts with G banding)	Death, day 1

polyhydramnios and fetal edema in two cases, with hydrops appearing in one fetus just before delivery, after normal ultrasonographic examinations at 20 and 27 weeks. All four infants required extensive ventilatory support with oxygen at birth. All had significant edema and ascites at birth. Evaluation ruled out renal, gastrointestinal, hematologic, and viral etiologies of the nonimmunologic hydrops fetalis. Features of Down's syndrome became evident after diuresis in the two patients who survived more than 2 days. The third infant had Down's syndrome (death on day 2) diagnosed by skin fibroblast cultures. The fourth infant died soon after birth and had multiple anomalies consistent with trisomy 13.

Comment

Chromosomal trisomy disorders are considered rare causes of nonimmunologic hydrops fetalis. Only 25 cases (trisomy 21, n = 17; trisomy 18, n = 6; trisomy 13, n = 2) have been previously reported. Analysis of previously reported series of nonimmunologic hydrops fetalis that included infants with chromosomal trisomies and the present study yields an overall incidence of 7% in live-born infants with nonimmunologic hydrops fetalis, with a range from 3%[1] to 29% in the present study. However, the incidence of chromosomal trisomies could be higher or lower since hydropic fetuses may die in utero. Hydrops fetalis has been noted in first- and second-trimester fetuses with trisomy 21, 18, and 13.[1, 2] The cause of edema in chromosomal trisomies is unknown. Fetal hypoxia due to placental abnormalities may result in capillary endothelial damage, leading to increased capillary permeability and subsequent edema formation.

The association between nonimmunologic hydrops fetalis and chromosomal trisomies has valuable clinical applications. When fetal edema is diagnosed via ultrasonography, amniocentesis and fetal karyotype determination should be considered. Hydropic fetuses may have at least a 7% chance of a chromosomal trisomy, and female fetuses have the additional risk of having an XO karyotype (Turner's syndrome). Chromosomal abnormalities should always be considered in the differential diagnosis of nonimmunologic hydrops fetalis in the fetus and neonate. Their features are often distorted by edema, thus obscuring the phenotypic characteristics of the common trisomies.

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Excretion of varicella–herpes zoster virus in breast milk

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Two cases involving breast-feeding and varicella–herpes zoster virus infection are presented. The possibility of viral excretion in the breast milk and its effects on continued breast-feeding are discussed. In neither case was the virus isolated from the breast milk. (AM J OBSTET GYNECOL 1986;154:1116-7.)

Key words: Lactation, varicella–herpes zoster virus, breast milk

Both chickenpox and herpes zoster infections occur during the reproductive years. It seems possible that a breast-feeding infant could acquire herpes zoster infection from an infected mother either by direct skin contact during nursing or by consuming virus-contaminated milk. An extensive search through clinical and basic science literature did not produce relevant data on the excretion of the varicella–herpes zoster virus in breast milk or on the clinical consequences of continued breast-feeding during a maternal infection. We recently saw one case of chickenpox and one case of herpes zoster dermatitis in lactating mothers. In both cases we monitored the method of feeding, the course of the disease in the mother, the possible presence of excreted virus in breast milk, and signs of herpes zoster infection in the infant.

Case reports

Case No. 1. A 33-year-old woman, gravida 5, para 3-0-2-3, 6 months post partum, lactating, and with a history of childhood chickenpox, presented with cutaneous vesicles involving the T4-5 dermatome on the left back and side. The breast was not involved. The lesions were painful grouped, classic herpetiform vesicles on an erythematous base, with patches varying in size from 2-4 cm. Vesicles progressed from clear to necrotic in the usual sequence during the next few weeks after the initial presentation. Within 24 hours of the appearance of the lesions, milk was expressed separately from each breast directly into culture tubes. Milk was cultured on diploid human fibroblasts and then examined for cytopathic effect. After the appropriate incubation period, the cultures proved negative for varicella–herpes zoster virus. The lesions were treated symptomatically and with topical acyclovir beginning at the time of presentation. On the sixth day after eruption crusting began, and by 2 weeks after eruption the lesions were healing. At the time of presentation the infant had been solely breast-fed with only recent introduction of solids. She had nursed on the affected side on the day lesions were first noted. Breast-feeding was allowed to continue, and position changes were used to avoid infant contact with lesions.

Case No. 2. A 21-year-old woman, gravida 2, para 1-0-0-1, presented at 40 weeks' gestation with chickenpox contracted from her older child. No prior history of chickenpox was noted. Her immediate course was complicated by severe pulmonary compromise due to varicella pneumonia. Emergency cesarean section was performed. A healthy infant was delivered and treated prophylactically with zoster immune globulin and parenteral acyclovir. The infant did not develop clinical evidence of chickenpox and was discharged after 10 days' observation. In spite of her complicated postpartum course, the mother expressed an interest in maintaining lactation potential by using a breast pump. Milk production was established and sustained for several weeks, during which time the breast milk was cultured by the same methods as in Case 1. Again no growth of virus was obtained. Because of the very serious nature of the pulmonary complications, the mother never actually put the infant to the breast, nor was the expressed breast milk given to the infant. At 3 weeks post partum she elected to stop pumping and was offered lactation suppression.

Comment

In the first case reported, it was possible to prevent direct lesion contact and to maintain breast-feeding. The milk in this case did not reveal virus and no infant infection occurred. In the second case, extensive cutaneous lesions prevented actual breast-feeding because of the danger of neonatal varicella infection from maternal contact.[1] These two cases suggest that breast-feeding can continue during maternal herpes zoster infection without risk of herpes zoster infection via virus-contaminated breast milk and that, while the mother with chickenpox at delivery must be isolated from her infant, it may still be possible to safely give

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her milk to her infant. Furthermore as maternal antibodies are excreted, anti–herpes zoster antibodies may be excreted in the milk, which help to further protect the infant from herpes zoster infection.[2] Additional data are needed to substantiate the safety of continued breast-feeding during maternal herpes zoster infection and of feeding expressed maternal milk from infected mothers to their infants.

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Folate deficiency presenting as pancytopenia in pregnancy

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Folate deficiency is a well-known complication of pregnancy. We present a case of folate deficiency with profound pancytopenia. Although this is a well-known consequence of folate deficiency, we wish to reemphasize this dramatic complication of pregnancy. (Am J Obstet Gynecol 1986;154:1117-8.)

Key words: Folic acid, pancytopenia, pregnancy

Pancytopenia is a well-known consequence of folate and vitamin B_{12} deficiencies. Vitamin B_{12} deficiency is distinctly unusual in pregnancy although levels may fall during the course of pregnancy secondary to hemodilution and vitamin B_{12} binders present in the serum. The incidence of folate deficiency varies from 0.2% to 30% during pregnancy depending on geographic locations and socioeconomic status. The routine use of folate supplementation has decreased the incidence in the United States. However, folate deficiency is the most common cause of macrocytosis in pregnancy. Hemoglobinopathies, hemolytic anemias, thyroid disease, and liver disease also cause macrocytosis.

Case report

R. D., a 21-year-old, gravida 2, para 1, white woman, presented at 38 weeks' gestation to Magee-Womens Hospital with ruptured membranes of 36 hours' duration. A routine blood count revealed pancytopenia with hemoglobin, 5.3 gm/dl; hematocrit, 16%; red blood cell count, 1.55×10^6; platelets, 33,000/mm^3; and a white blood cell count of 1900/mm^3 with 58% polymorphonuclear cells, 33% lymphocytes, 1% eosinophils, 2% bands, 1% metamyelocytes, 2% myelocytes, and 2% atypical lymphocytes.

The patient had an unremarkable past medical history. Her antepartum course was notable for persistent nausea and vomiting throughout the pregnancy. Hemoglobin and hematocrit at 18 weeks' gestation were 12.5 gm/dl and 36.8%, respectively. At 30 weeks, she continued to vomit and also had symptoms suggestive of reflux esophagitis with heartburn and an acid taste on lying flat and bending over. A repeat blood count was not performed. She claimed compliance with multiple vitamins, folate, and ferrous sulfate, which was standard prenatal care in the medical care center. From an obstetric standpoint, growth was appropriate by fundal height determination. From 18 to 30 weeks, she gained 4 kg; however, she gained only 1 kg from 30 weeks to delivery. At presentation she complained of fatigue and a sore tongue. She denied the use of antibiotics, anticonvulsives, and toxin exposure, and there were no symtpoms of malabsorption.

Physical examination revealed a well-developed woman with a yellow pallor in no acute distress. Vital signs included: blood pressure, 120/80 mm Hg; pulse, 120 bpm and regular; temperature, 37.8° C; respirations, 16/min. Skin was without petechiae. Sclera were icteric. Tongue was beefy red. No palatal petechiae or ulcerations were present. The chest was clear. The heart was tachycardiac with a Grade II/VI systolic ejection murmur. Abdominal examination was without hepatosplenomegaly. Fundal height was 38 cm, and fetal heart tones were 160 bpm. Mild contractions were occurring every 2 to 5 minutes. Cervix was 2 cm dilated at the −2 station with vertex presentation. Rupture of the membranes was confirmed. She did not have lymphadenopathy or sternal tenderness. Peripheral blood smear revealed hypersegmentation of polymorphonuclear cells with a seven-lobed nucleus. There was

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some anisocytosis and poikilocytosis with rare red blood cell fragmentation. Red blood cells were not grossly macrocytic on smear and on automated counter mean corpuscular volume was 103 and platelets were of normal size.

The initial impression was probable folate deficiency and concomitant iron deficiency, given the mild macrocytosis. She received four units of packed red blood cells and went into active labor. She was delivered of a viable female infant weighing 7 pounds, 12 ounces via spontaneous vaginal assist. Estimated blood loss was 700 ml. She had moderately severe postpartum atony. The infant had a hematocrit of 55% with a mean corpuscular volume of 120; white blood cells and platelets were normal.

Bone marrow examination of the mother 3 days post partum revealed a hypercellular marrow with megaloblastic myeloid predominance and decreased iron stores. Red blood cell folate was 64 μg/ml (normal 210 to 980 μg/ml); vitamin B_{12} level, 150 μg/ml (low normal); bilirubin, 3.2 mg/dl; direct bilirubin, 0.4 mg/dl; lactate dehydrogenase, 2627 units.

The patient received folate and ferrous sulfate. On discharge, white blood cell count was 15,900/mm^3; platelets, 117,000/mm^3; hemoglobin, 7.8 gm/dl; hematocrit, 23.8%; red blood cell count, 2.53 × 10^6; reticulocyte count went from 0.49% (6400/mm^3) to 5.4% (136,600/mm^3) during 5 days. Lactate dehydrogenase was 796 units. Six weeks post partum white blood cell count was 6000/mm^3; hemoglobin, 12.1 gm/dl; hematocrit, 36.6%; and platelet count, 324,000.

Comment

Folate requirements increase during pregnancy.[1, 2] Several authors estimate from 100 to 600 μg/day are needed to prevent folate deficiency in pregnancy. Placental and fetal needs coupled with the increased urinary excretion of folate are important in the genesis of deficiency. Gastrointestinal disturbances that occur during pregnancy can lead to dietary deficiency. A high-affinity binder of folate has been demonstrated in the serum of pregnant women as well.

We suspect that our patient did not exhibit profound macrocytosis from folate deficiency since she was also iron deficient. Generally one six-lobed polymorphonuclear white blood cell confirms the diagnosis of megaloblastic anemia. Both folate and vitamin B_{12} are required for deoxyribonucleic acid synthesis, and deficiency can lead to delayed synthesis that affects all cell lines and leads to resultant pancytopenia as in our patient.

Unfortunately, serum folate levels are not very useful in pregnancy because they tend to fall, yet the marrow is normoblastic. Red blood cell folate levels are somewhat more useful but may also be normal in the early phases of folate deficiency. Urinary formiminoglutamic acid excretion after histidine administration also has been useful in pregnancy. One must be cautious in the interpretation of vitamin B_{12} levels as in the present case. In addition to pregnancy, folate deficiency can also cause levels of vitamin B_{12} to be low because of overutilization of vitamin B_{12} in the absence of folate.

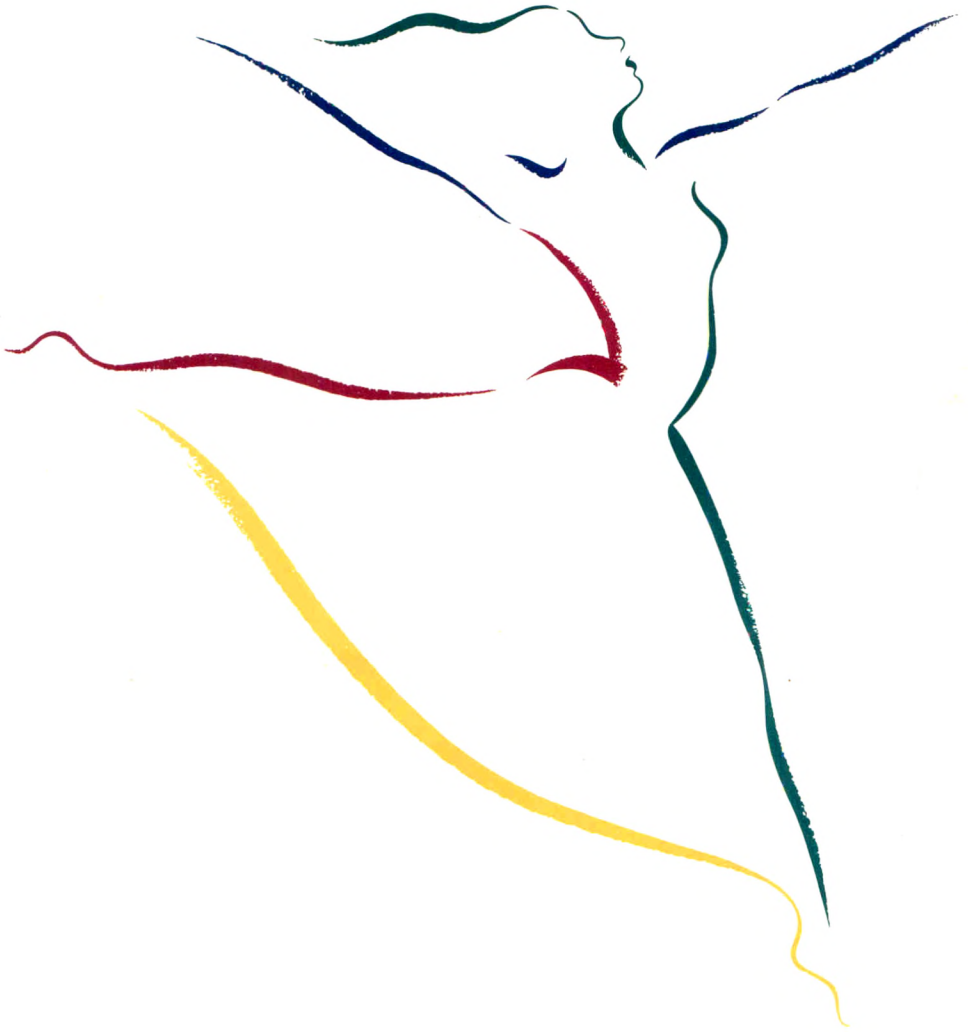
Folate deficiency has been linked to abruptio placentae, toxemia, neuropsychiatric syndrome, stillbirths, congenital anomalies, and decreased birth weights; however, subsequent studies have not supported these relationships. The role of folate in neural tube defects remains controversial.

Fortunately folate deficiency is on the decline in pregnant women with the use of prophylactic folate. Clinicians must still be watchful, particularly in patients with gastrointestinal disturbances of pregnancy, for the consequences of severe folate deficiency as occurred in our patient.

REFERENCES

1. Bitran JD, Miller B, Golomb HM. Megaloblastic anemia during pregnancy. J Reprod Med 1977;19:186-92.
2. Shojania AM. Folic acid and vitamin B-12 deficiency in pregnancy and in the neonatal period. Clin Perinatol 1984;11:433-59.

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1. According to a 12-month nationwide industry audit (ending October 1985), comprised of approximately 2,000 pharmacies. Vulvar burning occurred in 2.3% and itching in 0.9% of patients on Femstat.
2. Bradbeer CS, Mayhew SR, Barlow D: Butoconazole and miconazole in treating vaginal candidiasis. *Genitourinary Med* 61:270-272, 1985. (Study conducted in non-pregnant patients.)

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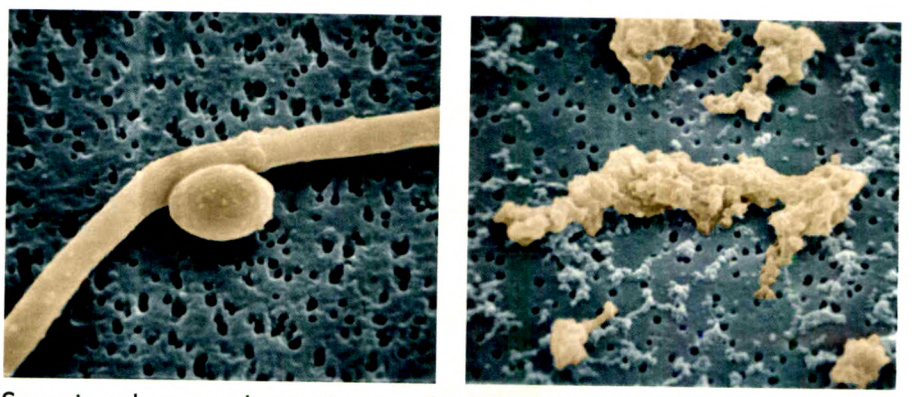
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FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

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Information for the Patient:
Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis:
Animal studies have not been done.

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Mutagenicity studies were negative.

Impairment of Fertility:
Animal studies showed no impairment of fertility.

Pregnancy Category C:
Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers:
Use with caution.

Pediatric Use:
Safety and efficacy have not been established.

ADVERSE REACTIONS:
Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

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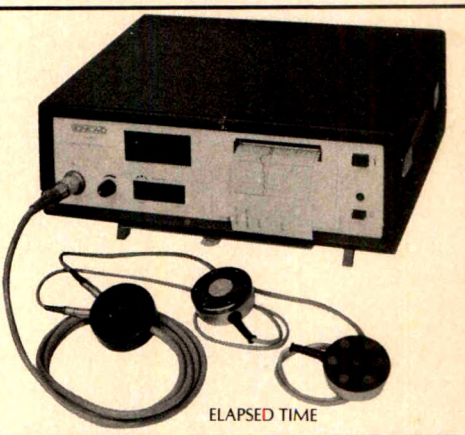
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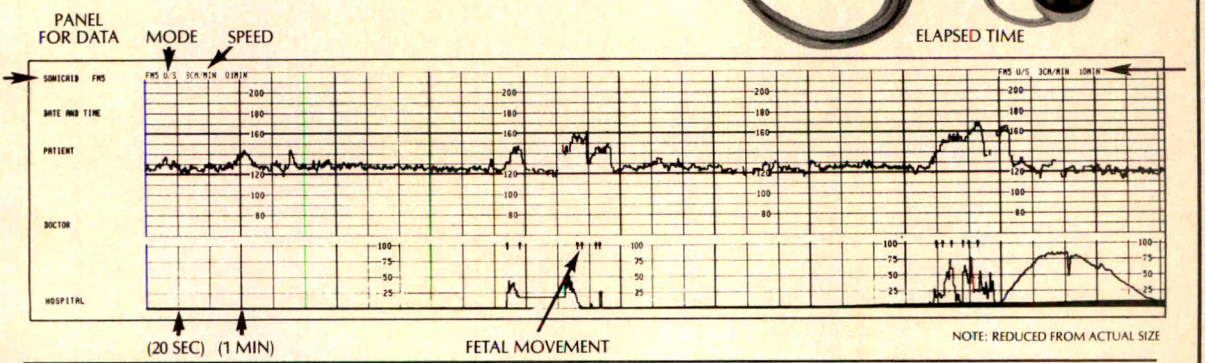
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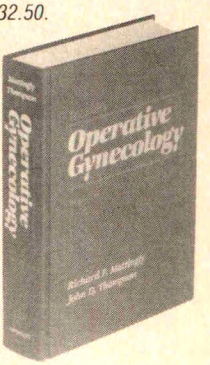
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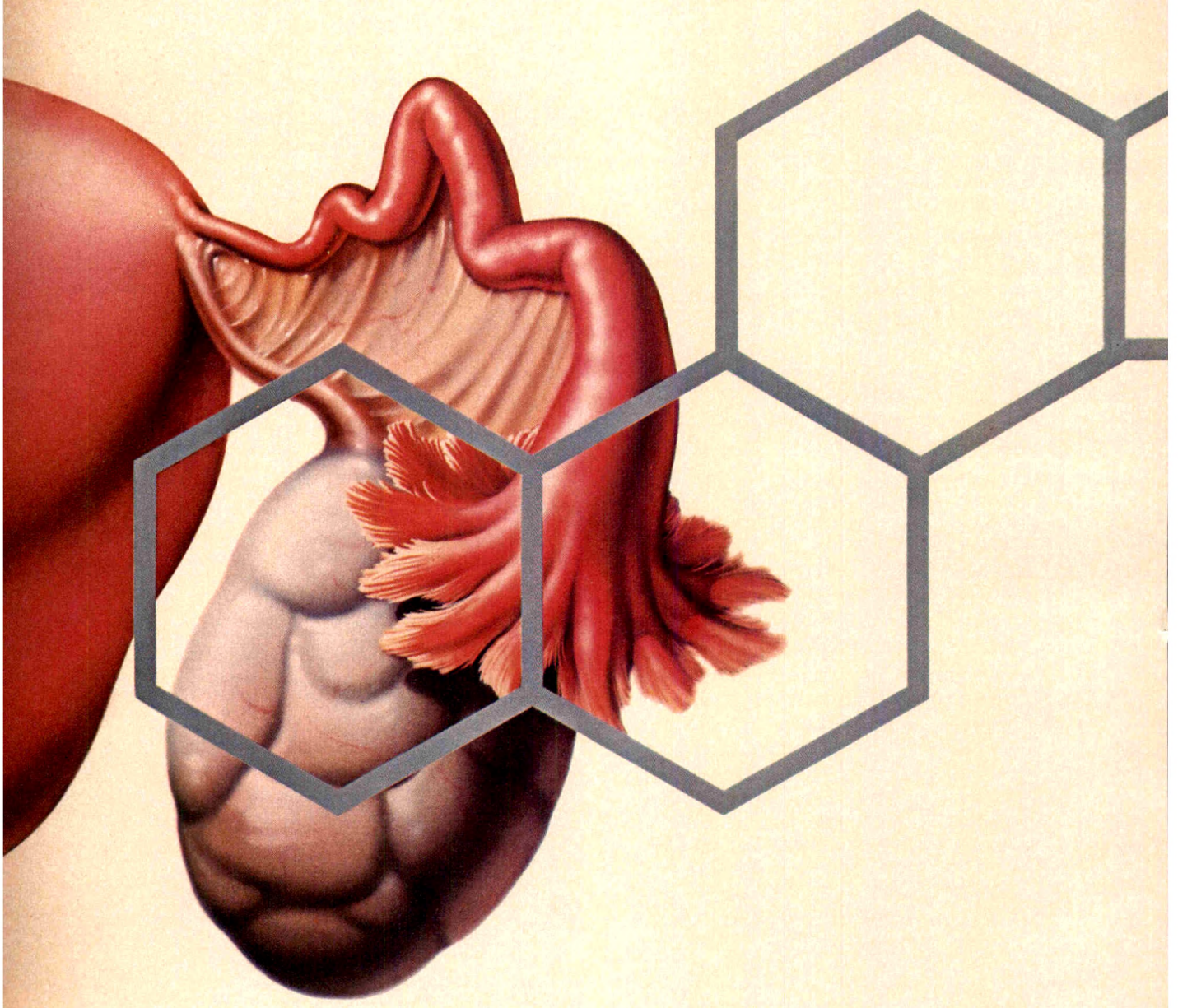
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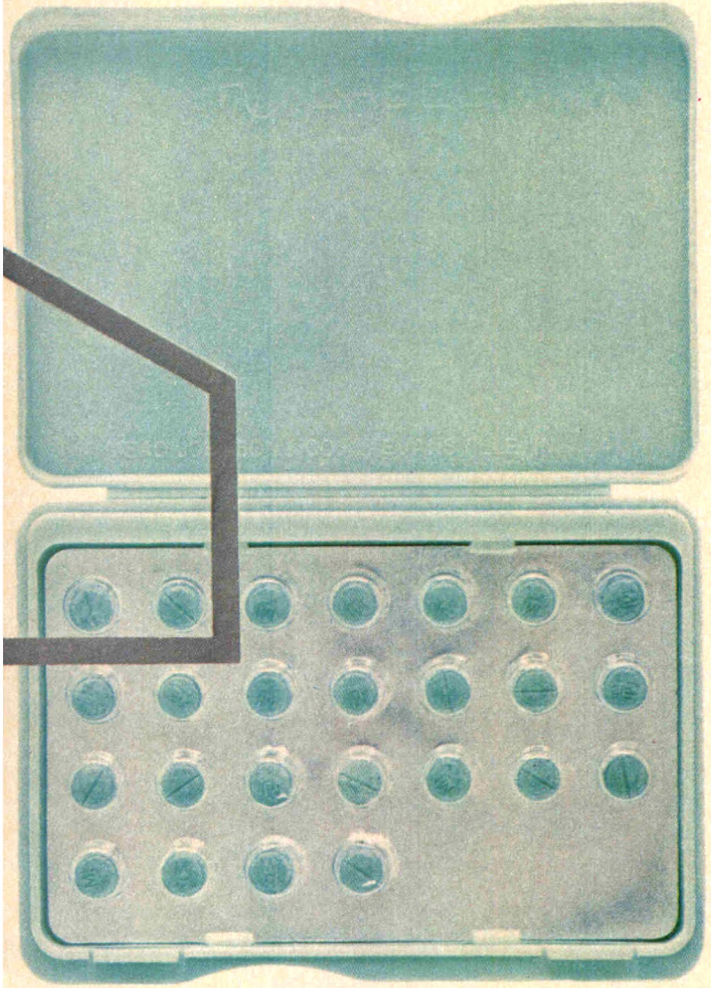
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2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

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Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Information for the Patient*—See text of Patient Package Insert. *Pregnancy Category X*—See Contraindications and Boxed Warning. *Nursing Mothers*—Administer to nursing mothers only when clearly necessary.

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Gaucher's disease in pregnancy associated with portal hypertension

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A case is reported of Gaucher's disease in pregnancy associated with portal hypertension. Despite this rare and hazardous complication, the pregnancy, labor, and puerperium were uneventful. (AM J OBSTET GYNECOL 1986;154:1119-20.)

Key words: Gaucher's disease, pregnancy, portal hypertension

Gaucher's disease is the most common inherited metabolic disorder of glycolipid metabolism. The disease is an inborn error of intracellular metabolism characterized by abnormal storage of cerebroside in the reticuloendothelial system. On reviewing the literature, we were unable to find any case in which ascites was associated with Gaucher's disease in pregnancy. We report here the first case of confirmed Gaucher's disease in pregnancy associated with portal hypertension. Despite this serious complication, the prenatal course was uneventful, with good fetal outcome.

Case report

Z. M., a 23-year-old Jewish woman (gravida 1, para 0), was admitted at 12 weeks' gestation to the antenatal high-risk unit for evaluation. Her past medical history revealed that at the age of 4 she was diagnosed as having Gaucher's disease. At the age of 5 years she underwent a splenectomy, and at the age of 12 she had a fracture of the right femur head. A review of all the other organ systems was normal. A complete study of the patient's family revealed no other members that suffer from the disease.

On admission, physical examination revealed a blood pressure of 110/70 mm Hg, pulse of 90 bpm, respirations of 12/min, and temperature of 36.4° C. The patient was well nourished and asthenic, with a greatly enlarged abdomen. The skin had a buckwheat pallor. There was no icterus or cyanosis. Examination of the head and neck revealed no significant abnormality. The heart and lungs were normal and there was no adenopathy. The abdomen was markedly protuberant and the umbilicus everted. Some superficial venous distention was noted. A fluid wave was easily elicited. The liver edge was palpable 10 cm below the right costal margin; it was firm and not tender. The legs showed brownish pigmentation characteristic of Gaucher's disease. Uterine size was consistent with 12 weeks' gestation. The cervix was posterior, firm, and closed.

Ultrasound examination indicated a single fetus with crown-rump length of 54.7 mm. The abdominal scan revealed a huge ascites with no other abnormality.

Laboratory findings were as follows: The hemogram revealed anemia with a hemoglobin of 8.8 gm/dl; hematocrit of 27%; white blood cell count of 10,000/mm^3 with normal differential count; prothrombin time of 11 to 13 seconds; partial thromboplastin time of 30 to 34 seconds; platelets of 190,000/mm^3; and fibrinogen level of 590 mg/100 ml. The fasting blood sugar level, liver enzymes, and levels of creatinine, uric acid, and electrolytes were within normal limits.

The patient received supplements of iron and folic acid; she remained ambulatory and was followed up until onset of spontaneous labor. Consecutive measurements of uterine size and repeated ultrasound scanning indicated normal fetal growth. Because of the greater risk and the serious nature of the complications, frequent clinic visits were made with special attention given to liver function. Except for anemia, no other hematologic or gastrointestinal complications were observed during pregnancy.

At 41 weeks of gestation, labor began spontaneously and after 10 hours, a live, healthy female infant, weighing 3440 gm, was delivered. During labor the mother was carefully monitored for hematologic complications with cross-matched blood available on standby. The third stage of labor was uneventful, with a normal postpartum course. Three days after delivery, both she and her baby were discharged in a satisfactory condition. X-ray examination of the upper gastrointestinal tract revealed enlarged esophageal varices.

Comment

Gaucher's disease in pregnancy is rare. Most authors have concluded that in cases of Gaucher's disease in

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pregnancy, if close monitoring is undertaken, one can expect a good fetal outcome with no adverse effect on the mother. The majority of affected patients have a chronic low-grade anemia, which can usually be controlled with hematinics and rarely may require blood transfusion. Recently, Goldblatt and Beighton[1] reviewed the medical aspects of 21 pregnancies accompanied by Gaucher's disease and concluded that there are a few contraindications to pregnancy and no increased incidence of infertility, abortions, stillbirths, or congenital anomalies.

Although hepatosplenomegaly is commonly found in patients with Gaucher's disease, clinical complications of liver disease have rarely been reported. In nonpregnant patients, bleeding from esophageal varices as well as portal hypertension and ascites has been noted. These findings may be attributed solely to the massive presence of Gaucher's cells in the liver. Obliteration of the central veins and sinusoids and compression of the portal triads by Gaucher's cells and connective tissue seem to be the cause of the portal hypertension.[2]

Although in patients with portal hypertension there is an increased probability of bleeding varices as pregnancy nears term, no such complication was seen here. It is now clear that there is no evidence to indicate that Gaucher's disease significantly affects the course and prognosis of pregnancy in any adverse way. The case reported here confirms this evidence. Despite Gaucher's disease complicated by portal hypertension, the pregnancy, labor, and puerperium were uneventful.

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Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography

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Fetal echocardiography is a well-established technique for the prenatal identification of congenital heart disease. One of the indications for its use is the presence of extracardiac anomalies, as such coexistent defects may have important implications for obstetric and neonatal management. We have reviewed the obstetric and pediatric literature to examine reported associations. If a fetus is suspected to have hydrocephalus, microcephaly, holoprosencephaly, agenesis of the corpus callosum, Meckel-Gruber syndrome, esophageal atresia, duodenal atresia, diaphragmatic hernia, omphalocele, or renal dysplasia, cardiac evaluation should be pursued. Furthermore, echocardiography may be of help in differential diagnosis of some anomalies (for instance, skeletal dysplasias). Maternal diabetes and phenylketonuria, as well as exposure to phenytoin, trimethadione, or isotretinoin, may result in multiple systemic defects, including congenital heart disease. (AM J OBSTET GYNECOL 1986;154:1121-32.)

Key words: Fetal echocardiography, extracardiac anomalies, congenital heart disease, prenatal diagnosis

High-resolution two-dimensional ultrasound has provided physicians with a tool for the prenatal diagnosis of malformations arising in virtually all parts of the body. A wide variety of diagnoses have been established from the gastrointestinal tract and the genitourinary, musculoskeletal, and central nervous systems.[1, 2] The information obtained can be directly applied for parental counseling and planning appropriate perinatal management.[3]

These techniques have been extended to the study of the heart as well.[4-7] At present, fetal echocardiography is directed toward pregnancies considered at particularly high risk of congenital heart disease[8]; the most frequent indication in our laboratory is a previous infant with congenital heart disease.[9] In these cases the risk of congenital heart disease is between 2% and 5%.[10] Fetal echocardiography has also been recommended for the evaluation of any fetus with extracardiac malformations.[8, 11]

Some extracardiac malformations carry a high risk of associated congenital heart disease, while for others the risk is low. We decided to examine those associations reported in the past in both the obstetric and pediatric literature, to help clarify this indication for the use of fetal echocardiography. The following categories will be considered: systemic anomalies, genetic syndromes, twins, and syndromes arising from selected environmental agents and from maternal metabolic diseases.

Most of our knowledge about the association of congenital heart disease with extracardiac malformations comes from studies of children identified because of cardiac abnormalities. Fewer studies have considered the reverse, that is, the incidence of congenital heart disease when extracardiac malformations have been identified. We shall review this issue from both sides. Significant differences may be anticipated in the findings of various studies depending on the method of case ascertainment. If the anomalies present are severe enough to cause a high perinatal mortality rate, these cases will probably be removed from consideration in pediatric studies. Conversely, an autopsy-based study may disclose clinically inapparent defects.

Systemic extracardiac malformations

The overall incidence of extracardiac malformations in children identified as having congenital heart disease

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Table I. Incidence of associated systemic malformations in propositi with congenital heart disease

System	Series					
	Greenwood et al.[12]		*Gallo et al.*[14]		*Wallgren et al.*[13]	
	n	%	*n*	%	*n*	%
Central nervous	107/1566	6.9	44/1354	3.2	60/1000	6.0
Respiratory	58/1566	3.8	109/1354	8.0	52/1000	5.2
Gastrointestinal	65/1566	4.2	*		155/1000	15.5
Genitourinary	83/1566	5.3	*		131/1000	13.1
Musculoskeletal	137/1566	8.8	62/1354	4.5	161/1000	16.1
Total	395/1566	25.2	446/1354	32.9	439/1000	43.9

*Original report contained overlap between subgroups.

varies from 25% to 45% (Table I).[12-14] This variation appears to depend on the means of identification, with a lower incidence in clinical studies than in autopsy studies. The incidence also appears to be related to the type of cardiac defect identified.

Greenwood et al.[12] found that in abnormalities such as atrioventricular septal defects extracardiac malformations were present in more than 50% of cases, while atrial septal defects, ventricular septal defects, tetralogy of Fallot, and cardiac malpositions were associated with extracardiac malformations in about 30% of cases. They found a similarly high association with patent ductus arteriosus, although the frequency with which this occurs among otherwise normal premature infants makes it difficult to interpret the significance of this observation. While the other defects listed are all potentially diagnosable antenatally, the ductus is normally open in utero, and we shall not consider this further.

The second group Greenwood et al. identified included coarctation of the aorta, single ventricle, isolated pulmonary stenosis, and double-outlet right ventricle. Each of these was associated with extracardiac malformations in about 20% of cases. Additionally, the combination of pulmonary atresia with a ventricular septal defect was associated with extracardiac malformations in 18% of cases. All of the other structural congenital heart disease identified was associated with extracardiac malformations in <15% of cases.

Greenwood et al. additionally considered possible patterns of association between specific defects. They identified some trends, which we shall consider as we examine each system.

Central nervous system. The association of central nervous system lesions with congenital heart disease is relatively infrequent (Table I). Wallgren et al.,[13] in a study of 1000 children with congenital heart disease in a combination of clinical and autopsy findings, reported that 6% had associated central nervous system malformations. Of the 60 affected infants, 22 had unspecified brain anomalies, 17 had hydrocephalus, 12 had a meningomyelocele, and 17 had abnormalities of the sensory organs (eyes or ears). No specific pattern of relationships was found between the types of congenital heart disease and central nervous system abnormalities.

Greenwood et al.[12] found central nervous system abnormalities in 6.9% of 1566 infants with congenital heart disease, while Gallo et al.[14] reported an association in 3.2% of 1354 cases. Neither provided details concerning the types of central nervous system lesions present. Greenwood et al. failed to identify any specific pattern of congenital heart disease, but Gallo et al.[14] found an excess of ventricular septal defects (39%) among their population of 44 patients with congenital heart disease and central nervous system abnormalities.

Few clinical studies are available concerning the incidence of congenital heart disease in infants with congenital abnormalities of the central nervous system (Table II). In a series of 205 cases of isolated congenital hydrocephalus, nine cardiac malformations were found (4.5%).[15] Congenital heart disease, mainly ventricular septal defects, has occasionally been reported in association with Dandy-Walker syndrome[16, 17] and holoprosencephaly.[18] The latter association may be related to the frequent presence of concurrent trisomy 13.[19]

Congenital heart disease was observed in 15% of 47 patients found to have agenesis of the corpus callosum, with no specific pattern detected.[20] No specific association has been described between congenital heart disease and failure of neural tube closure (anencephaly, spina bifida), porencephalia, or hydranencephaly. As the causes of microcephaly are heterogeneous, including chromosomal aberrations, viral infections, and environmental agents (for instance, phenytoin, alcohol), congenital heart disease may often be seen.

A complex syndrome involving the presence of microcephaly, often with an occipital encephalocele, polycystic kidneys (Potter type III), polydactyly, cleft palate, and ambiguous genitalia is referred to as the Meckel syndrome (or Meckel-Gruber syndrome). The exact incidence of congenital heart disease is uncertain; however, Opitz and Howe[21] found a 14% incidence in a review of the previous literature. It is inherited in autosomal recessive fashion.

In a series of 27 cases of prenatally diagnosed hy-

drocephalus, Chervenak et al.[22] found congenital heart disease in four: two ventricular septal defects and one case each of tricuspid hypoplasia and tetralogy of Fallot. In our experience of 31 cases of isolated hydrocephalus, three cardiac abnormalities were seen: one pulmonary atresia with an intact ventricular septum and two atrioventricular septal defects.[23]

In summary, about 5% of cases of hydrocephalus diagnosed postnatally have associated congenital heart disease, and 10% to 15% of cases of hydrocephalus diagnosed prenatally have congenital heart disease. Central nervous system abnormalities are seen overall in 3% to 7% of infants identified as having congenital heart disease. Series of prenatally diagnosed congenital heart disease are too small to permit conclusions to be drawn and are biased by the fact that the central nervous system abnormality itself may be the indication for the fetal echocardiogram.

Mediastinal and respiratory defects. Structural defects of the trachea and esophagus are often concomitant, because of their close embryologic association; we shall consider them together with respiratory system anomalies. The association of these lesions with cardiac and noncardiac malformations is well-known and has been considered the consequence of a common insult, possibly environmental. Many acronyms have been applied to these syndromes (VATER: vertebral, anal, tracheoesophageal, radial, and/or renal; VACTEL: vertebral, anal, cardiac, tracheoesophageal, limbs; and VACTERL: a combination of the previous two).

Series of patients with tracheoesophageal fistula and/or esophageal atresia have shown congenital heart disease to be present in 15% to 40% of cases (Table II). An excess of atrial and/or ventricular septal defects has been reported in two series[24, 25] but has not been consistently noted, as shown in Table III.[26, 27] In one series, the presence of other extracardiac malformations was shown to have a strong influence on the likelihood of congenital heart disease; there was only an 8.3% incidence in isolated tracheoesophageal fistula or esophageal atresia, but the incidence rose to 22% if another extracardiac malformation was present.[27] The presence of congenital heart disease had a significant influence on mortality as well.

Wallgren et al.[13] reported 47 cases of esophageal atresia in 1000 infants with congenital heart disease; the incidence of tracheoesophageal fistula was not presented. Gallo et al.[14] found 14 cases of esophageal atresia in 1354 cases of congenital heart disease (1.0%) but also did not report the frequency of tracheoesophageal fistula. Among 1566 infants with congenital heart disease, Greenwood and Rosenthal[27] found tracheoesophageal fistula or esophageal atresia in only eight cases (0.5%), five of which were associated with other anomalies as well.

The most common type of cardiac defect found with esophageal atresia consisted of ventricular septal de-

Table II. Incidence of associated congenital heart disease in propositi with systemic malformations

System or lesion	*Frequency of congenital heart disease*	
	n	%
Central nervous system		
Hydrocephalous		
Burton[15]	9/205	4.5
Chervenak et al.[22]*	4/27	14.8
Pilu[23]*	3/31	9.7
Dandy-Walker syndrome†		
Sawaya and McLaurin[16]	1/23	4.3
Hirsch et al.[17]	1/40	2.5
Agenesis of corpus callosum[20]	7/47	14.9
Meckel-Gruber syndrome[21]	5/36	13.8
Neural tube defects	NA	
Microcephaly	NSP	
Holoprosencephaly[18]	CR	
Mediastinum		
Tracheoesophageal fistula		
Mehrizi et al.[24]	13/39	33.3
Esophageal atresia–tracheoesophageal fistula		
Landing[25]	11/28	39.2
Mellins and Blumenthal[26]	48/184	26.0
Greenwood and Rosenthal[27]	48/326	14.7
Gastrointestinal		
Duodenal atresia		
Fonkalsrud et al.[29]	86/503	17.1
Jejunal atresia		
DeLorimier et al.[30]	12/233	5.2
Anorectal anomalies		
Teixeira et al.[32]	15/68	22.0
Imperforate anus		
Greenwood et al.[31]	26/222	11.7
Ventral wall		
Omphalocele		
Greenwood et al.[40]	31/159	19.5
Carpenter et al.[41]	8/25	32.0
Gastroschisis		
Seashore[39]	0/25	0
Carpenter et al.[41]	1/13	7.7
Diaphragmatic hernia		
Greenwood et al.[45]		
Own data	11/48	22.9
Review	33/345	9.6
Genitourinary		
Greenwood et al.[46]†		
Renal agenesis		
Bilateral	3/7	42.8
Unilateral	12/71	16.9
Horseshoe kidney	7/18	38.8
Renal dysplasia	2/37	5.4
Ureteral obstruction	1/48	2.1
Others	5/272	1.8
Total	30/453	6.6

NA = No specific association found in literature. NSP = No specific association demonstrable because of multiple etiologies. CR = Only case reports available; exact incidence cannot be estimated but a greater than chance association appears to be present.

*Prenatal series.

†Excludes cases of patent ductus arteriosus.

Table III. Cardiac defects associated with tracheoesophageal fistula and/or esophageal atresia

Series	Septal defects			Other defects	Total congenital heart disease	
	Atrial	Ventricular	Both		n	%
Landing[25]	2	5*	3	1	11/28	39
Mellins and Blumenthal[26]	20	15†	2	11	48/184	26
Greenwood and Rosenthal[27]	5	23‡	—	20	48/326	15
Mehrizi et al.[24]§	2	5*	2	4	13/39	33

*Includes one patient with tetralogy of Fallot.
†Includes three patients with tetralogy of Fallot.
‡Includes six patients with tetralogy of Fallot.
§Tracheoesophageal fistula only.

fects, present in 12 of 47 (25.5%) of the series of Wallgren et al.[13] and 12 of 48 (25.0%) of that of Mellins and Blumenthal.[26] This is similar to the expected incidence of ventricular septal defect in an unselected population of children with congenital heart disease and may not represent a specific association.

Anomalies of the respiratory system were present in 5% of the cases reported by Wallgren et al.,[13] with hypoplastic lungs responsible for 19 of 52 (36.5%). Other lung abnormalities, primarily lobar, were the cause of an additional 25 cases, with 10 more caused by malformations of the trachea and larynx and choanal atresia. Greenwood et al.[12] found unspecified respiratory system anomalies in 58 of 1566 (3.8%) cases of congenital heart disease. The incidence of respiratory tract anomalies found by Gallo et al.[14] was similar, with anomalies present in 109 of 1354 (8.0%) cases of congenital heart disease.

Gastrointestinal system. The major forms of gastrointestinal abnormality diagnosed in the prenatal period are the bowel atresias. Of these the proximal obstructions tend to present earliest, as they are more likely to cause detectable increases in amniotic fluid volume or dilation of the stomach. Duodenal atresia is associated with trisomy 21 in 30% of cases. Touloukian[28] further reported that four of five infants with both trisomy 21 and duodenal atresia had malformations of the atrioventricular septum. Fonkalsrud et al.[29] noted that 17% of infants with duodenal obstruction had coexisting congenital heart disease. On the other hand, congenital heart disease is only found in 5.2% of infants with jejunal or ileal atresia (Table II).[30] This difference may result from the varying causes of the lesions. The duodenum is a solid core of tissue that undergoes vacuolization and recanalization between the fourth week and the sixth week of development. Failure of recanalization is often seen in association with abnormalities of pancreatic and terminal biliary development. Jejunoileal and colonic atresias are most often the result of ischemic injury occurring after the tenth week, when the gut has returned to the coelomic cavity.[28]

There have been wide variations reported in the frequency of association between imperforate anus and congenital heart disease (Table II). Greenwood et al.[31] reported a 12% incidence of congenital heart disease, most commonly tetralogy of Fallot and ventricular septal defects. Teixeira et al.,[32] in contrast, found that of 68 patients with anorectal anomalies, 15 (22%) had congenital heart disease, although 13 of these had other systemic and/or chromosomal anomalies. Three of their patients, all with multiple anomalies, had isolated patent ductus arteriosus, while an additional three patients had Ivemark syndrome (asplenia with congenital heart disease, see below). These figures may overestimate the true incidence, as congenital heart disease has also been reported in only 5.6% of patients with anorectal malformations.[33]

The overall incidence of intestinal malformations and obstructions in infants with congenital heart disease is 2% to 7% (Table I).[12-14] Wallgren et al.[13] found 22 cases of imperforate anus among their 1000 patients with congenital heart disease. Greenwood et al.[12] found that 12 of 1566 patients with congenital heart disease had imperforate anus (0.8%).

The heterotaxia syndromes (Ivemark syndrome, cardiosplenic syndrome) include the combination of congenital abnormalities of the spleen (asplenia or polysplenia), variable degrees of visceral heterotaxia, and complex congenital heart disease. Asplenia is usually associated with right atrial isomerism, and severe congenital heart disease is found in virtually all patients, frequently consisting of atrioventricular septal defects, often in association with transposition of the great arteries and anomalous pulmonary and systemic venous return. In this syndrome, the viscera take on a "bilateral right-sidedness" with bilateral trilobed lungs, and a midline liver with malrotation of the gut.[34]

Polysplenia syndrome is more often associated with "bilateral left-sidedness." There is left atrial isomerism, often with anomalous systemic (interrupted inferior vena cava with azygous continuation) and pulmonary venous return and atrioventricular septal defects.[34]

Identification of this syndrome is based on careful

evaluation of the visceral site as well as the segmental cardiac anatomy.[35] Prenatal diagnosis of heterotaxia syndrome has recently been reported,[36] and we have seen several examples in our laboratory as well.

It is important to differentiate this syndrome from Kartagener syndrome (immotile cilia syndrome; bronchiectasis, dextrocardia, and sinusitis), which is inherited in autosomal recessive fashion and appears to be associated with cardiac anomalies only if situs ambiguus is present.[33] Kartagener and Stucki[37] reviewed 116 cases of the syndrome and found only two with congenital heart disease, both having situs ambiguus. Holmes et al.[38] found congenital heart disease in two of 13 cases, but at least one of these had associated absence of the spleen.

Ventral wall defects. Defects of the anterior abdominal wall are traditionally divided into two groups based on the pathophysiologic features and resultant location of the defect. Gastroschisis is a full-thickness defect of the abdominal wall, usually occurring to the right of the umbilicus. It has been suggested to arise as a result of a localized failure of mesenchymal development with consequent ectodermal necrosis and evisceration. Complete nonrotation of the gut is invariably present.[39] It does not appear to be associated with an increased risk of congenital heart disease (Table II).

In contrast, omphalocele is one of a number of malformations of the abdominal wall that occurs because of failure of the normal infolding process of the borders of the embryonic disc. If this occurs low on the abdominal wall, involving the caudal fold, exstrophy of the bladder will result. If it is high, involving the cephalic fold, thoracic ectopia cordis is present along with sternal and diaphragmatic defects. If only the lateral folds are affected, then the intestines fail to return to the peritoneal cavity, and an umbilical defect covered by an amnioperitoneal membrane will result.

Omphalocele is associated with congenital heart disease in 19% to 32% of cases (Table II).[40, 41] The incidence of congenital heart disease seems to relate to which of the embryonic folds are involved. If only the caudal fold is involved, the heart is rarely affected. Greenwood et al.[40] found that 10% of neonates with lateral fold defects had congenital heart disease, with the frequency rising to 100% if the cephalic fold was affected. The most frequent defect was tetralogy of Fallot, which was present in 32% of patients with congenital heart disease.

The frequent association of upper midline defects with congenital heart disease has been given the eponym pentalogy of Cantrell. The full syndrome includes the presence of (1) a midline supraumbilical abdominal defect, (2) a defect of the lower sternum, (3) deficiency of the diaphragmatic pericardium, (4) deficiency of the anterior diaphragm, and (5) a congenital intracardiac abnormality.[42] In a literature review, Toyama[43] found a total of 36 cases of Cantrell syndrome, of which 18 (50%) had atrioventricular septal defects, six (18%) had ventricular septal defects, and four (11%) had tetralogy of Fallot.

The association of omphalocele with visceromegaly, macroglossia, and severe neonatal hypoglycemia has been termed Beckwith-Wiedemann syndrome. It has been reported to be associated with congenital cardiovascular malformations in 12 of 13 cases (92%). Seven of these 12 (58%) had structural congenital heart disease, while the other five had isolated cardiomegaly. No specific pattern of congenital heart disease could be determined.[44]

Diaphragmatic defects. Most congenital diaphragmatic defects occur through the foramina of Bochdalek or Morgagni. They may mimic congenital heart disease in and of themselves because of secondary cardiac malposition or vascular compression. There is also reported to be a significant association with true structural heart disease (Table II). Greenwood et al.[45] found that 11 of 48 patients with diaphragmatic hernia had associated congenital heart disease (22.9%). In a review of the literature before that report, they found a 9.6% (33 of 345) incidence. Greenwood's series included two patients with anterior herniation in association with omphalocele, one with tetralogy of Fallot, the other with ectopia cordis and intrinsic structural heart disease (possible Cantrell pentalogy), and two other patients with Down syndrome, which may account for the higher incidence in that series. Greenwood also noted, in a review of 1566 infants with congenital heart disease, that five (0.3%) had associated diaphragmatic hernia.

Genitourinary system. Consideration of associations between congenital heart disease and genitourinary malformations is complicated by the broad spectrum of disease entities encompassed by this group. Certain anomalies, such as unilateral obstruction of the ureter either at the renal pelvis or at the pelvic brim, are relatively benign. Others, such as bilateral renal agenesis, are catastrophic in and of themselves, while others, such as horseshoe kidneys and various genital malformations, are often associated with a wide variety of additional systemic abnormalities and chromosomal aberrations.

In their review of 1566 children with congenital heart disease, Greenwood et al.[12] found that 83 (5.3%) had associated genitourinary anomalies. Gallo et al.[14] noted that urinary anomalies were present in 110 of 1354 (8.1%) children with congenital heart disease and that 57 (4.2%) genital anomalies were present. No mention was made of whether any overlap existed between these two groups. Wallgren et al.[13] found genitourinary anomalies in 131 of 1000 patients with congenital heart disease (13.1%). Of these 131 patients, there were 30 (23%) with hydronephrosis, 24 (18%) with renal agenesis, 15 (11%) with horseshoe kidney, 28 (21%) with genital malformations, and 56 (43%) with other anom-

Table IV. Incidence of congenital heart disease in propositi with skeletal anomalies

Syndrome	*Inheritance pattern*	*Frequency of congenital heart disease (%)*
Short rib–polydactyly (3 types)[49, 50]	Autosomal recessive	Common*
Chondrodysplasia punctata[10, 51]	Autosomal recessive	Uncommon
Thrombocytopenia–absent radii[52]	Autosomal recessive	33
Fanconi[53]	Autosomal recessive	14
Crouzon[10, 33]	Autosomal dominant	Uncommon
Apert[54]	Autosomal dominant	10
Carpenter[55]	Autosomal recessive	3.2
Robin[33, 56]	Mixed	9
Arthrogryposis[57]	Mixed	Common†
Holt-Oram[58]	Autosomal dominant	Common‡
Ellis-Van Creveld[10]	Autosomal recessive	50

The terms common and uncommon are used in situations where the literature provides only approximations of risk.

*Case reports only in literature.

†Heterogeneous nature of diagnosis makes exact assignment of risk difficult. Some kindreds reported to have congenital heart disease in 25% of affected individuals.[57]

‡Congenital heart disease invariably present in complete form of disease, usually present as well in various formes fruste of the syndrome.

alies. They also noted that 24% of patients with coarctation of the aorta and 21% of those with ventricular septal defect had associated genitourinary anomalies.

In examining 453 infants with genitourinary malformations, Greenwood et al.[46] found congenital heart disease in 34 (8%). This association was especially prominent among the seven patients with bilateral renal agenesis, five of whom had congenital heart disease (71%). Two of these, however, had patent ductus arteriosus, which might be anticipated in a neonate with persistently high pulmonary arterial pressures and arterial hypoxemia due to concomitant pulmonary hypoplasia. Neonates with horseshoe kidney, a condition not yet diagnosed in utero, were found to have congenital heart disease in 44% of cases. No other associations between specific genitourinary and cardiovascular anomalies were apparent.

Skeletal system. Anomalies of the skeleton can be diagnosed in utero by ultrasound[47] and are often associated with cardiac anomalies. Greenwood et al.[12] found that 137 of 1566 infants (8.8%) identified as having congenital heart disease had associated skeletal abnormalities. Wallgren et al.[13] found such an association in 161 of 1000 children with congenital heart disease (16.1%), with an excess of tetralogy of Fallot (27% of affected infants). These figures are difficult to interpret, however, because of the heterogeneity of diagnoses represented.

Table IV provides information concerning skeletal dysplasias known to be associated with an increased risk of congenital heart disease. Some of these anomalies are quite rare, making the reports of associated cardiovascular abnormalities open to ascertainment bias. Further detail concerning the diagnostic criteria for the various syndromes may be found in sources devoted to the subject.[33, 48]

Genetic syndromes

Although many of the systemic associations we have mentioned above are inherited with recognized Mendelian patterns, there is another group of disorders that are conveniently described as genetic syndromes. We shall consider these in two groups: those related to chromosomal abnormalities and those inherited as syndromes with common patterns of abnormality but no grossly identifiable chromosome defect. From the latter group we have already considered those associated with skeletal dysplasias, as well as the Meckel-Gruber syndrome.

Chromosomal aberrations. A number of chromosomal defects are characteristically associated with congenital heart disease. Any list of such associations is necessarily incomplete as many autosomal abnormalities result in first-trimester spontaneous abortion, and accurate evaluation of cardiac structures, or primordia, is rarely undertaken. Table V presents relevant information about known associations between chromosomal abnormalities and congenital heart disease. It should be noted that each of the syndromes listed is accompanied by many additional somatic abnormalities, complete cataloging of which is beyond the scope of this report. For further details the interested reader is referred to texts dedicated to this subject.[10, 59]

Given the presence of a fetus with congenital heart disease, it is important to be aware that the risk of a coexistent chromosomal abnormality is approximately 5%.[10, 60] Since many of the phenotypic abnormalities associated with these anomalies are not detectable by ultrasound alone, an amniocentesis should be offered to all patients whose features have congenital heart disease.

Mendelian inheritance. The Noonan syndrome encompasses features of Turner syndrome (such as short

stature, characteristic facies, hypertelorism, webbed neck, and shield chest) but in the presence of a normal karyotype. These phenotypic features may not be diagnosable in utero; however, congenital heart disease is found in 50% to 55%,[10, 33] with right heart involvement in 80% of these, usually pulmonary valvular or infundibular stenosis. Asymmetric left ventricular free-wall hypertrophy may be seen[61] and should be amenable to diagnosis in utero, if present at the time of examination. Tetralogy of Fallot, Ebstein anomaly, ventricular and atrial septal defects, and total anomalous pulmonary venous return have also been described.[61, 62] As the syndrome is transmitted as autosomal dominant, couples at risk may request prenatal diagnostic testing, and fetal echocardiography may be useful.

Twin pregnancies

An excess of monozygotic twins has been noted in a retrospective review of congenital heart disease among offspring of multiple births. Anderson[63] looked at 107 children identified because of the presence of congenital heart disease in patients who were also twins. He found that of the 107 sets of twins, 67 were monozygotic (63%) and that there was a higher concordance rate for congenital heart disease among those judged to be monozygotic (6.8%) than among those judged to be dizygotic (2.2%; similar to the expected concordance rate for nontwin siblings). It is difficult to determine from this review whether the actual incidence of congenital heart disease is higher among twins than among singletons, but it would appear that of the overall population with congenital heart disease seen by Anderson, one of 63 cases were twins, which is in the same range as the frequency of twins in the general population. Schinzel et al.[64] identified a number of congenital anomalies that were more prevalent among monozygotic twins but did not include congenital heart disease.

There was a disproportionate number of monozygotic twins among the propositi of Anderson's population, leading him to conclude that there may be an increased tendency toward congenital heart disease among monozygotic twins.[63] Myrianthopoulos[65] reported that twins had a 17.5 per 1000 risk of congenital heart disease, as compared with 7.57 per 1000 among singletons. Although specific figures are not included, most of the increase was among monozygotic twins.

The rarest form of monozygotic twinning is conjoined twins, estimated to occur in from one per 30,000 to one per 100,000 pregnancies.[66] Of these, approximately 45% are either thoracopagus or thoracoomphalopagus twins, with omphalopagus representing another 10%. When the chest wall is shared, congenital heart disease is present in about 75%, and in most of the remainder the pericardium is shared.[67] The most frequent form of congenital heart disease is a conjoined heart with two ventricles and a varying number of atria, although a number of different permutations of numbers of atria and ventricles have been reported.[68] In virtually all, septal defects are present in addition to other deformities.[67] Even in those with omphalopagus and sharing no thoracic structures, congenital heart disease has been described in at least one of the twins in more than a quarter of twin pairs. It is interesting to note that only one of nine sets reported had concordance of the congenital heart disease in both twins.[69]

Another rare form of twinning is the development of acardia, estimated to occur in 1% of monozygotic twins, or one per 35,000 births. There appears to be a spectrum of disease ranging from the true acardius (holoacardius), in which no cardiac tissue is present, to the pseudoacardius, in which a rudimentary heart is present.[70] The parasitic twin may cause the heart of the normal twin to develop a volume-overload type of congestive heart failure, which has been successfully treated in utero with maternal digitalization.[71]

Drug-related malformations

A number of drugs have been implicated as the primary cause of various syndromes of congenital malformations, often including the heart. Now only of historic interest, thalidomide was primarily known for its association with phocomelia when ingested in the first trimester. If the exposure was between days 20 and 36 after conception, a 5% to 10% incidence of congenital heart disease has been identified, especially tetralogy of Fallot, truncus arteriosus, and septal defects.[72]

The fetal alcohol syndrome, consisting of mental retardation, microcephaly, intrauterine growth retardation, hypertelorism with other facial abnormalities, and joint anomalies, has usually been seen in women consuming >60 ml of absolute alcohol per day, but more moderate alcohol consumption may carry some risk as well.[59] Congenital heart disease has been identified in 25% to 30% of infants with the full syndrome, especially ventricular and atrial septal defects.[72-74] While the joint anomalies and facial abnormalities, such as the hypoplastic philtrum, may be difficult to identify by ultrasound, some of the others, such as intrauterine growth retardation, microcephaly, hypertelorism, and cleft lip, could be recognized.

Approximately 0.5% of pregnant women have epilepsy,[59] so the obstetrician is often faced with women who require anticonvulsants during the period of organogenesis. All studies of the potential teratogenicity of anticonvulsants are hampered by the unknown effect of untreated maternal epilepsy on development. The lack of association between phenobarbital and any malformation syndromes, however, speaks for a relatively small contribution, if any, from the maternal disease. One study[75] of the effects of phenytoin has suggested that women treated sporadically in the first trimester,

or only in the latter two trimesters, had a rate of malformation (about 30 per 1000) intermediate to that of those with no disease (24 per 1000) and those treated throughout gestation (61 per 1000). Several other studies have compared untreated epileptic patients with the general population and found no difference in overall malformation rates.[76-78]

The fetal hydantoin syndrome includes growth retardation, microcephaly, hypertelorism, a short broad nose, and hypoplasia of the nails and distal phalanges. Congenital heart disease has been described in 2% to 3%, particularly ventricular septal defect, pulmonary stenosis, aortic stenosis, and coarctation of the aorta.[72]

Trimethadione is less commonly encountered, with the primary indication for its use being petit mal epilepsy. It has been reported to be associated with a high incidence of congenital heart disease, with as many as 15% to 30% of exposed fetuses found to have transposition of the great arteries, tetralogy of Fallot, or hypoplastic left heart. Other features of fetal exposure are craniofacial and genital abnormalities, as well as intrauterine growth retardation.[72, 79, 80]

Exposure of the fetus to sex steroid hormones as a cause of congenital heart disease has been a source of controversy in the literature. An association between maternal unspecified sex hormone use and multiple anomalies, including cardiovascular, skeletal, gastrointestinal, and genitourinary, was first suggested by Nora et al.[81, 82] Harlap et al.[83] and Levy et al.[84] found similar effects in larger cohorts of patients.

Combination oral contraceptives were suggested by Heinonen et al.[85] to cause an increase in the rate of congenital heart disease from 7.8 per 1000 in a control group to 21.6 per 1000 (an adjusted relative risk of 2.4). Estrogens alone were associated with an adjusted relative risk of 1.4 and progestins alone with an adjusted relative risk of 1.5, with a cumulative risk for these two agents alone or in combination of 2.1. No unusual patterns of congenital heart disease syndromes were noted in these prospectively collected data, although others have suggested a relative increase in conotruncal defects,[74] nor were associated extracardiac anomalies found. A similar relative risk of congenital heart disease was found in a retrospective study performed by Rothman et al.,[86] although again no pattern of specific malformations was found and the 90% confidence limits for the incremental risk were 1.0 to 2.1.

It has been suggested that progesterone derivatives are associated with limb reduction defects,[87] as well as other components of the VACTERL syndrome,[81, 88] although other investigators[89] have failed to reproduce these findings.

The data are somewhat contradictory, but a twofold increase in congenital heart disease appears to be present in fetuses exposed to combination oral contraceptives or sex steroid analogues.

The most recent addition to the list of possible cardiac teratogens is the vitamin A analogue isotretinoin (Accutane, Roche Laboratories). A survey conducted by the American Academy of Dermatology and the United States Food and Drug Administration found 35 instances of pregnancy exposure, with resultant spontaneous abortion or congenital anomalies in 29 (83%). Those with congenital anomalies had a consistent pattern of central nervous system anomalies, primarily hydrocephalus and posterior fossa cysts, along with micrognathia and abnormal ears. In addition, five of the 10 anomalous infants had congenital heart disease, especially septal defects and great artery abnormalities. Exact risks are hard to assign as the true number of exposed fetuses is unknown. Some bias is inevitable because of selective reporting, but the defects were remarkably similar between infants and similar as well to those abnormalities seen in experimental animals.[90]

Maternal metabolic diseases

There are two classical maternal diseases associated with an elevated risk of fetal anomalies, phenylketonuria and diabetes mellitus. In both, preconceptual counseling and strict control during early organogenesis, before most pregnancies are even clinically recognized, are essential in minimizing the risk of congenital malformations.

Patients with phenylketonuria may safely relax their dietary regimens in adolescence without adverse effects. It has recently become evident that hyperphenylalaninemia during organogenesis results in a number of malformations. Intrauterine growth retardation, microcephaly, and congenital heart disease are all prominent, and all appear in increasing frequency and higher maternal phenylalanine levels.[91] When maternal levels exceed 15 mg/dl, the frequency of congenital heart disease has been reported to be 12% to 16%.[92] Tetralogy of Fallot is disproportionately common among these children. It is important for phenylketonuric women to be aware of the small elevation in plasma phenylalanine that occurs after ingestion of aspartame (Nutrasweet, Searle Laboratories).[93]

The incidence of congenital heart disease is increased fivefold among infants of diabetic mothers.[94] The overall frequency of congenital anomalies has been related to first-trimester hemoglobin A_{1c} levels, an indicator of long-term glucose levels.[95] Ventricular septal defects and transposition of the great arteries are the most common forms of congenital heart disease among these infants. Hypertrophic cardiomyopathy may occur if control is poor in the latter part of gestation as well, although this may be expected to regress in the newborn period.[96] Other anomalies that may be detected by ultrasound include neural tube defects, renal anomalies, and the caudal regression syndrome.[97]

Table V. Congenital heart disease and chromosomal syndromes

Chromosomal aberration	*Live-born incidence*	*Congenital heart disease incidence (%)*	*Typical lesions*
Trisomy 21 (Down)	1/800	50	VSD, CAVC
Trisomy 18 (Edward)	1/8,000	99	VSD, CAVC, DORV
Trisomy 13 (Patau)	1/20,000	90	VSD
Trisomy 8	Rare*	50	VSD
Trisomy 22	Rare	67	ASD
Trisomy 22†	Rare	40	TAPVR
Trisomy 9	Rare	>50	VSD
45, X (Turner)	1/5,000	35	Coarct, Bic AOV
5p- (Cri Du Chat)	1/20,000	20	VSD
4p- (Wolf)	Rare	40	ASD
13q-	Rare	25	VSD
+14q-‡	Rare	>50	PDA
18q-	Rare	<50	VSD
XXXXY	Rare	14	PDA§
Triploidy	Rare	‖	VSD

Adapted from Nora and Nora[10] and Simpson et al.[59]

VSD = Ventricular septal defect; CAVC = complete atrioventricular canal; DORV = double outlet right ventricle; ASD = atrial septal defect; TAPVR = total anomalous pulmonary venous return; Coarct = coarctation of the aorta; Bic Aov = bicuspid aortic valve; PDA = patent ductus arteriosus.

*"Rare" means not detected among prospective live-born surveys. In these syndromes, incidence of congenital heart disease is based on case reports.

†Partial trisomy 22 (cat-eye syndrome).

‡Partial trisomy 14q.

§Significance is uncertain, as it represents one of six reported cases.[10]

‖Quite rare among live births, making assessment of congenital heart disease risk impossible.

Intrauterine infections

The classic example of viral teratogenesis is the rubella syndrome, which may include cataracts, deafness, and microcephaly in addition to congenital heart disease. Infection may cause abortion in up to 50% of fetuses infected in the first trimester and other effects in 70% of the remainder. Among those with congenital heart disease, patent ductus arteriosus is present in 58%, ventricular septal defect in 18%, tetralogy of Fallot in 7%, and pulmonic stenosis in 6%.[10, 59, 60] Although women inadvertently vaccinated during early gestation have been shown to have virus cultured from the products of conception at abortion,[98] no teratogenic effects of vaccination have been found.[99]

An association between certain enteroviruses and congenital heart disease was suggested in one study based on early and late pregnancy serologic testing, particularly coxsackieviruses B3 and B4.[100] Others have disputed these findings.[10]

Comment

We have reviewed associations between extracardiac congenital anomalies that may be identified with ultrasound and similarly identifiable cardiac malformations. When a nonfatal extracardiac malformation is identified, the presence of major structural heart disease will influence the long-term survival, and prior knowledge of its presence will permit optimal obstetric and neonatal management.

Fetal echocardiography has been successfully applied in the accurate evaluation of the structurally abnormal heart in a number of laboratories.[11, 101, 102] We feel that echocardiography should ideally be performed in all fetuses identified as having extracardiac malformations. At present, there are a limited number of laboratories with extensive experience in fetal echocardiography. Consultation with such centers may be advisable when anomalies known to have a high association with congenital heart disease are found.

Based on our review of the literature, we suggest that the anomalies found in Table IV represent those with a particularly high frequency of association with congenital heart disease. If such anomalies are identified in utero, fetal echocardiography should be sought, as the information obtained may have a significant impact on subsequent care. Future epidemiologic or clinical studies may result in modification of this list.

Fetal echocardiography can take another role in clarifying certain prenatal diagnoses. In a fetus with a high risk of a syndrome involving multiple systems due to genetic factors (for instance, family history of Noonan syndrome) or environmental exposures (for instance, maternal drugs, metabolic disorders, or infections), an abnormal fetal echocardiogram may provide a sentinel sign for other anomalies. When there are ambiguous anomalous findings in other systems (for instance, skeletal dysplasias) an abnormal fetal echocardiogram may assist in differential diagnosis.

Aneuploidies are known to carry a high risk of congenital heart disease (Table V). For many of the major

Table VI. Antenatal sonographic diagnoses that should prompt cardiac evaluation

Abnormalities of cardiac position
Central nervous system
Hydrocephalus
Microcephaly
Agenesis of the corpus callosum
Encephalocele (Meckel-Gruber syndrome)
Mediastinal
Esophageal atresia
Gastrointestinal
Duodenal atresia
Situs abnormality
Ventral wall
Omphalocele
Ectopia cordis
Diaphragmatic hernia
Renal
Bilateral agenesis*
Dysplastic kidneys
Twins
Conjoined
Monoamniotic†

*Uniformly fatal; presence of congenital heart disease unrelated to prognosis.

†Possible relationship to congenital heart disease; firm association not established.

chromosomal aberrations (tetraploidies, trisomy 13, or trisomy 18), the presence of congenital heart disease has little impact on long-term prognosis. For others, such as Turner syndrome (45,X), fetal echocardiography may provide important information for parental counseling and perinatal care.

Our current protocol when performing fetal echocardiography for any indication is to establish that fetal growth parameters are normal for the anticipated gestational age and to survey the fetal anatomy. This includes identification of the stomach, kidneys, bladder, cerebral ventricles, integrity of the spine, placental position, and morphologic features and adequacy of amniotic fluid volume. For this reason our team includes both obstetricians and cardiologists.

When a cardiac malformation is diagnosed, formal Level II scanning is undertaken to examine these other structures in detail. Because of the 5% risk of chromosomal abnormality in fetuses with congenital heart disease, we offer genetic amniocentesis to all patients unless delivery is imminent.

Similarly, whenever an extracardiac anomaly is found in our Level II obstetric ultrasound facility, we perform fetal echocardiography (Table VI). Through this sort of patient surveillance we will maximize the identification of those patients with intercurrent problems. This, in turn, will enhance our ability to plan optimal perinatal and neonatal management.

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Fetal microcirculation of abnormal human placenta

I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus

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The latex injection–corrosion cast technique coupled with scanning electron microscopy was applied to the study of small for gestational age placentas. The main ultrastructural changes observed in the fetal vasculature of small for gestational age placentas were less branching of arteries and veins, capillaries with variable diameters, and many capillary bud projections; also numerous "H"-shaped anastomoses were present in the capillary network. The corrosion/cast technique approach enabled us to visualize the three-dimensional distribution of arteries, veins, and capillary network as well as the ultrastructural alterations among the various-sized vessels in the abnormal placenta. Light microscopic examination of these placentas revealed numerous syncytial knots and fibrinoid deposits pressing on arteriolar and capillary vessels at different locations. We suggest that the numerous capillary buds and anastomoses are characteristics of neovascularization and may be a compensatory phenomenon of the capillary network. This information could shed new light on alterations of physiologic functions of the pathologic placenta. (AM J OBSTET GYNECOL 1986;154:1133-9.)

Key words: Fetal microcirculation, placenta, scanning electron microscopy, intrauterine growth retardation, neovascularization

Since the placenta is involved in the basic functions of metabolism, transfer of nutrients, exchange of gases, and endocrine secretion, it is essential for maintaining pregnancy and promoting normal embryonic-to-fetal development. An examination of the three-dimensional distribution and ultrastructural appearance of normal fetal placental vasculature helps in understanding these physiologic functions. The morphologic characteristics of fetal blood vessels in the human placenta have recently been examined by the latex injection–corrosion cast technique coupled with the scanning electron microscope.[1-3]

The latex injection–corrosion cast technique, when applied to the study of the pathologic small for gestational age (SGA) placenta, revealed new data on the ultrastructural appearance of abnormal fetal-placental vasculature. The purpose of the present study was to elucidate the ultrastructural changes in the fetal-placental vasculature that are present in the pathologic SGA placenta by scanning electron microscopy.

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Material and methods

Seven human placentas from mothers who were delivered of SGA babies (less than tenth percentile in weight at birth) were used for the study (Table I). The same latex injection–corrosion cast technique used in the study of normal human placentas was applied to this investigation.[3] The umbilical cord was threaded by a small catheter in the umbilical artery and vein and provided a route of entrance for the injection medium and a route of egress. After catheterization, the placenta was suspended in water and then flushed with warm saline at 50 mm Hg injection pressure. This was followed by an injection of 70% Cementex (Cementex Corp., New York, NY 10013). After polymerization by air drying, the latex inside the blood vessel hardened into a vascular cast. For further hardening, tissue was left in 10% formalin for 2 weeks. Then the placental tissue was macerated by treatment with sodium hypochlorite. The resulting vascular cast was air dried and

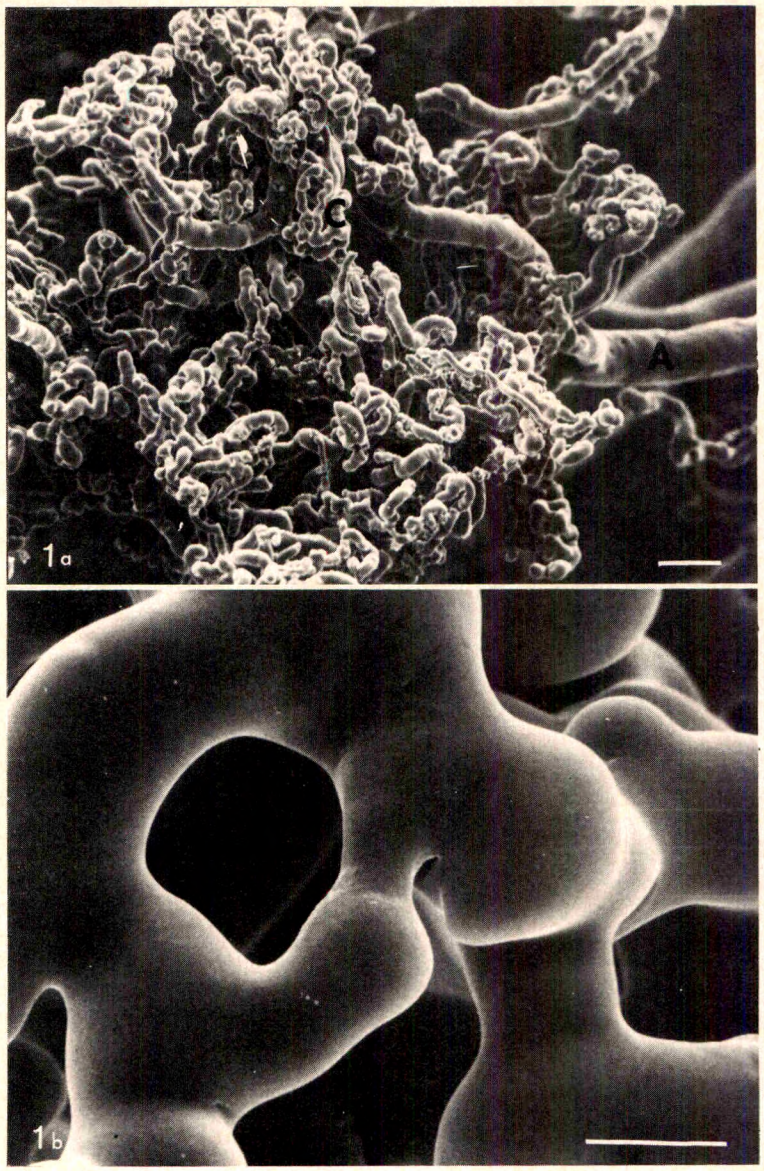


Fig. 1. *a*, Low-magnification scanning electron micrograph of cast from fetus with intrauterine growth retardation. An arteriole is shown on the right *(A)* leading into a fairly dense capillary network *(C)* located on the left. *Bar* = 100 μm. *b*, Scanning electron micrograph taken at high magnification of the capillary vessels in the placenta of an SGA fetus. Several H-shaped anastomoses are shown. *Bar* = 10 μm.

Table I. Clinical information

Patient	*Age (yr)*	*Gravidity and parity*	*Gestational age (wk)*	*Sex of baby*	*Weight (gm)*	*Mode of delivery*	*Apgar scores*	*Complications*
B. M.	24	G2P0	40	Female	2500	Cesearan section	6-9	Oligohydramnios
M. R.	27	G1P0	37½	Female	1500	Cesearan section	9-9-9	Hypertension
L. F.	27	G5P2	38½	Female	2040	Vaginal	8-9	
M. K.	27	G6P4	38	Female	2250	Vaginal	8-9-9	
S. F.	37	G2P2	37	Female	2400	Cesarean section	8-9	
C. L.	20	G1P0	31	Male	1000	Cesarean section	7-8-8	Severe pre-eclampsia
J. H.	37	G3P0	39½	Female	2210	Cesarean section	8-8-8	Meconium, positive CST

CST = Contraction stress test.



Fig. 2. Latex cast of the capillary network in the placenta of an SGA fetus. Note the numerous anastomoses and the presence of frequent vascular bud projections *(arrows)*. *Bar* = 10 μm.



Fig. 3. Scanning electron micrograph taken at low magnification of latex cast from fetal-placental vasculature in a fetus with intrauterine growth retardation. The central vessel is a vein with collecting venules. The vein has very few branches. *Bar* = 100 μm.

teased into small pieces for examination by scanning electron microscopy. The details of the injection–corrosion cast technique have been described in previous articles.[3-5]

For examination by scanning electron microscopy, the casts were attached to aluminum stubs by silver conductive paint and then coated with gold-palladium in a Hummer I Sputterer (Anatech, Ltd., Alexandria, VA 22310). On completion of the preparations, the resulting vascular casts were viewed and photographed on a JEOL-25 scanning electron microscope at 15 kV and 0- to 45-degree tilt.

A small portion of placenta was removed for regular light microscopic examination. The placental tissue was put into formalin and remained there for 2 weeks before processing for light microscopy. The sections for

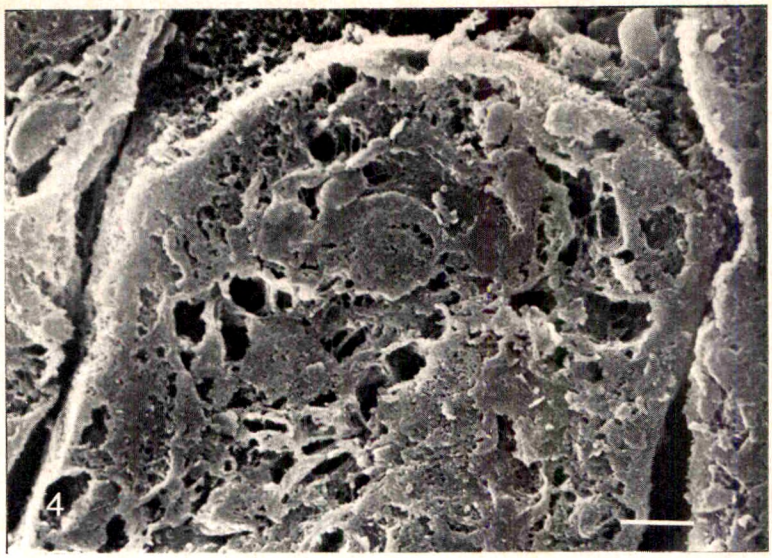


Fig. 4. An area of chorionic villi from an SGA placenta shows a number of capillaries that have compressed or occluded lumina. *Bar* = 10 μm.

light microscopy were cut at 8 μm and stained with hematoxylin and eosin for observation and photography.

Observations

Scanning electron microscopic observations of vascular casts from SGA placentas revealed the following alterations from normal morphologic features: the capillary network was more dense and contained many "H"-shaped anastomoses (Fig. 1 *a* and *b*); the capillaries were of variable diameters and contained numerous budding projections (Fig. 2); also less branching occurred among the arteries and veins than is found in normal human fetal-placental vessels (Fig. 3). Regarding the H-shaped capillary anastomoses, we found that they were numerous in the SGA placental microcirculation. The capillary network appeared more dense and more tortuous than that of the normal human placenta (Fig. 1, *a*). Furthermore, the number of fused loops was definitely increased over normal. While the number of capillary anastomoses increased, the amount of arterial and venous branching was decreased as compared with that of the normal placenta.

Scanning electron microscopy of whole-surface SGA placental tissue showed that large portions of the chorionic villi had occluded capillaries (Fig. 4) while a more normal-appearing villous capillary network area was seen elsewhere (Fig. 5). Also seen in the pathologic specimen were areas of fibrinoid-like material compressing portions of capillaries; many of the lumina of capillaries appeared collapsed or clogged. The umbilical cord from our SGA placenta was cut and processed for scanning electron microscopy. Fig. 6 shows a cross section of the cord with one patent umbilical vein and two umbilical arteries. One of the arteries is patent while the other has an occluded lumen; this was observed in two of the three umbilical cords.

Light microscopic observations of the pathologic placental tissue revealed that the chorionic villi had numerous syncytial knots (Fig. 7) on the villous surface. The cytotrophoblast was distinct while the syncytiotrophoblast was so thinly attenuated that it was not clearly seen. Fibrinoid deposits of variable size were observed around some villous capillaries. The villi themselves had a nonedematous appearance by light and scanning electron microscopy.

Comment

Vascular anatomy of the abnormal placenta with fetal growth retardation has been investigated by many researchers.[6-11] However, there have been no previous reports on vascular cast studies from abnormal placentas by scanning electron microscopy. This approach enabled us to visualize the three-dimensional distribution of arteries, veins, and capillary network in the pathologic placenta as well as the ultrastructural alterations among the various-sized vessels in the abnormal placenta. In addition, this approach could elucidate the alterations of the physiologic functions of the pathologic placenta.

Scanning electron microscopic analysis of fetal placental vascular casts revealed the following new findings: The capillaries have frequent H-shaped anastomoses and numerous forming vascular buds. Both phenomena are involved in neovascularization and may represent efforts by the abnormal placental capillary network to increase its density so that the fetus will have an increased capacity for exchange of nutrients and

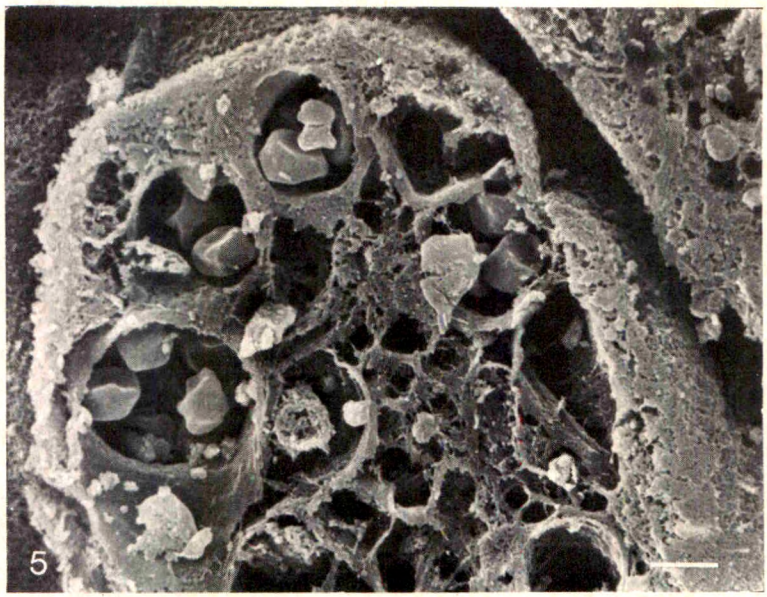


Fig. 5. A section of the chorionic villi from another portion of the SGA placenta showing a more normal-appearing cluster of capillaries with patent lumina, many of them filled with red blood cells. *Bar* = 10 μm.

gases at the capillary level. Neovascularization was similarly observed in vascular casts by scanning electron microscopy by Burger et al.[12] in the rat corneal capillary network after injury to the cornea by chemical cauterization. After 1 day, the injected vascular cast showed formation of new vascular buds from capillaries and venules. After a long time, microscopy of the casts revealed that a rich anastomosing capillary network had formed from the earlier vascular buds. The findings of Burger et al. are in conformity with our findings from placental capillary casts with regard to the frequent H-shaped anastomoses and frequent vascular bud formation. Although the placental type of neovascularization was not the result of chemical injury, it had a very similar ultrastructural appearance. The stimulus for the capillary neovascularization in a SGA placenta may be the initially reduced number of blood vessels in the placenta. This would impair nutrient and gaseous exchange for the developing fetus. Under this condition, an increase in the density of the capillary network would result in improving placental-fetal exchange. Presently we are working on an SGA animal model to explore this hypothesis.

From light microscopic examinations of these placentas, numerous syncytial knots and fibrinoid deposits (Kirby et al.[2]) were observed pressing on the arteriolar and capillary vessels in the chorionic villi. These findings may account for the variability of capillary diameters and some arteriolar constrictions as seen in the latex casts by scanning electron microscopy. The cause of the fibrinoid deposits is not yet fully understood, but McCormick et al.[13] and Fox[14] have suggested an immunologic source. Additional studies for further exploration of this possible mechanism may be warranted.

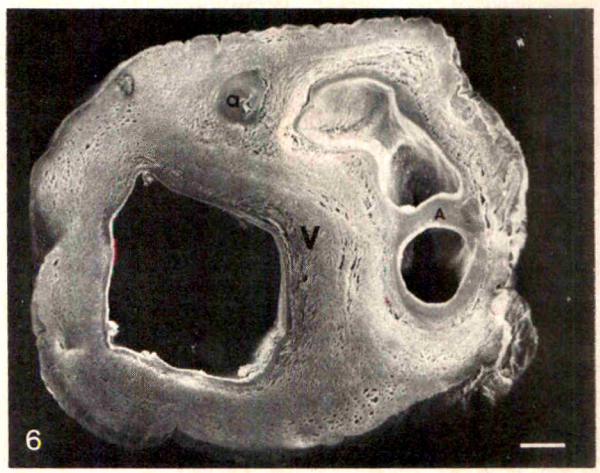


Fig. 6. Scanning electron micrograph of the umbilical cord from the placenta of an SGA fetus. The umbilical vein *(V)* is dilated, while one of the umbilical arteries *(A)* is patent and the other artery *(a)* is occluded. *Bar* = 1 mm.

Also seen by light microscopy were hyperplasia of the cytotrophoblastic cells and a thinly attenuated syncytiotrophoblastic layer. This finding confirms the light microscopic observations of Van Der Veen and Fox.[11] Normally at term the cytotrophoblast cannot be so clearly seen by light microscopy; such large, distinct cells probably represent highly active cells. Perhaps a genetic signal to these cells to reduce their functioning activity as term approached was lost or a time sequence of development was delayed. Another possibility is that

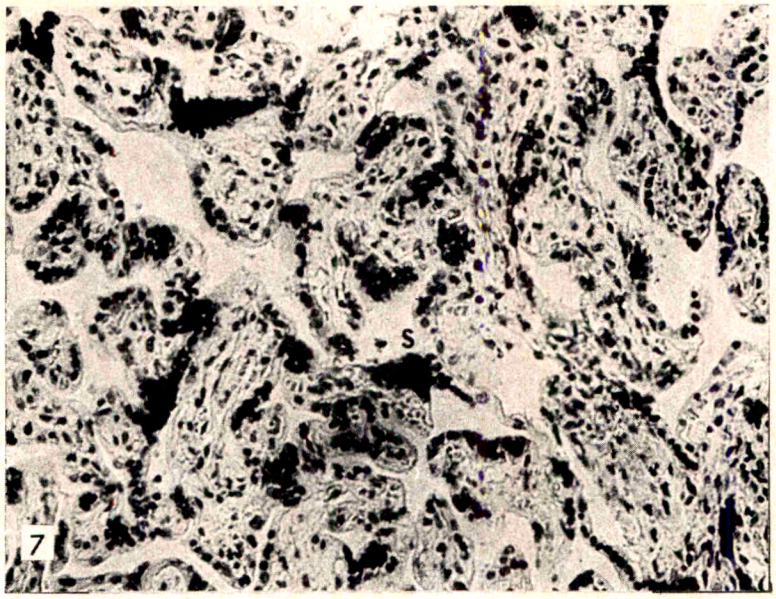


Fig. 7. Light micrograph of a section of the chorionic villi from an SGA placenta showing numerous syncytial knots *(S)* on the villi. The cytotrophoblast is distinct, while the syncytiotrophoblast layer is not clearly seen. (Hematoxylin and eosin. ×225.)

the hyperplasia of the cytotrophoblastic cells could be a compensatory repair mechanism. Reduced branching of the large vessels (arteries and veins) was also observed, which may indicate that there was an arrest of development during an early stage of placental blood vessel formation. With fewer large vessels, the volume of blood exchange to the growing fetus is decreased. The capillary network would thereby try to compensate by forming more capillaries to help increase the amount of surface area for nutrient and gaseous exchange. Our vascular casts of the term placenta of course cannot reveal the reason for a possible interruption of early development. We favor the concept that some event occurring early in blood vessel development caused alterations in the macrocirculation and microcirculation.

Cross section of the umbilical cord from an SGA placenta showed that while the umbilical vein appeared normal, the umbilical arteries had one patent and one occluded lumen. A possible explanation of this peculiar arrangement of one patent and one occluded umbilical artery is that there was a reduction of the amount of blood returning to the fetus, and the fetus became hypovolemic. As a consequence of this condition, both umbilical arteries cannot be maintained in the patent condition.

Our present scanning electron microscopic observations of latex casts provide an ultrastructural description of the alterations seen in the pathologic placentas, and light microscopy done in parallel supports the scanning electron microscope observations. In the future, transmission electron microscopy of specific vascular areas of the pathologic placentas will provide further ultrastructural data on the changes already observed by the other microscopic techniques.

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Fetal microcirculation of abnormal human placenta

II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis

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The latex injection–corrosion cast technique coupled with scanning electron microscopy was applied to the study of severe Rh sensitization of the placenta. The main observed ultrastructural changes in fetal vasculature in this type of placenta were very similar to those of the small for gestational age placenta; large-sized vessels exhibited less branching than those of the normal placenta, and the capillary network showed numerous vascular buds and "H"-shaped anastomoses. In addition, in Rh-sensitized placentas, the capillary network was remarkably sparse, and the cast also revealed smooth-surfaced veins with distinct U shapes undulating through the placental stroma. These U-shaped veins may be the result of umbilical flow impedance with subsequent umbilical venous hypertension, which stretches the veins into undulating form. (Am J Obstet Gynecol 1986;154:1139-46.)

Key words: Fetal microcirculation, placenta, scanning electron microscopy, Rh sensitization, neovascularization

The latex injection–corrosion cast technique coupled with scanning electron microscopy enables investigators to examine vascular casts from normal[1, 2] and abnormal human placentas in a three-dimensional form and to observe the ultrastructural changes in the fetal-placental vasculature. Several investigators[4-7] have described, using light and transmission electron microscopy, the appearance of Rh-incompatible placentas. In part one data were presented on ultrastructural changes observed in vascular casts from the placentas of small for gestational age fetuses.[3] This article will describe the vascular alterations that occurred in the placentas of fetuses with severe erythroblastosis fetalis and will discuss possible effects on the fetal-placental microcirculation resulting from such changes. To date, there has not been a scanning electron microscopic analysis of corrosion casts from Rh-incompatible placentas.

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Material and methods

Four placentas from Rh-sensitized mothers who had given birth to babies with severe anemia were used for the study (Table I). The same latex injection–corrosion cast technique used in the normal human placenta was applied to this investigation.[2] The umbilical cord was threaded by a small catheter into the umbilical artery and vein, providing a route of entrance of injection medium and a route of egress. After catheterization, the placenta was suspended in water, flushed with warm saline solution at an injection pressure of 50 mm Hg, and followed by an injection of 70% Cementex (Cementex Corp., New York, NY 10013). After polymerization by air drying, the latex inside the blood vessel hardened into a vascular cast. For further hardening the tissue was left in 10% formalin for 2 weeks. Then the placenta was macerated by treatment with sodium hypochlorite. The resulting vascular cast was air dried and teased into small pieces for examination in the scanning electron microscope as previously described.[2, 8]

For scanning electron microscopic examination, the casts were attached onto aluminum stubs by silver conductive paint and then coated with gold-palladium in



Fig. 1. *a*, Scanning electron micrograph of fetal arterial cast from the placenta of a fetus with erythroblastosis fetalis. This low-magnification photograph shows several arteries *(A)* with very few branches. *Bar* = 100 μm. *b*, Scanning electron micrograph of fetal capillary and venous vessels from the placenta of a fetus with erythroblastosis fetalis. The capillary network in this region is sparse and not densely coiled; a venule is shown on the lower left area. *Bar* = 100 μm.

a Hummer I Sputterer (Anatech, Ltd., Alexandria, VA 22310). On completion of the preparations, the resulting vascular casts were viewed and photographed on a JEOL-25 scanning electron microscope at 15 kV and 0- to 45-degree tilt.

A small portion of placenta was set aside for routine light microscopic examination. The placental tissue was put into formalin and remained there for 2 weeks before processing for light microscopy. The sections for light microscopy were cut at 8 μm and stained with hematoxylin and eosin for observation and photography.

Observations

Scanning electron microscopic observations of vascular casts from severe Rh-sensitized placentas showed that there was less branching of the large vessels than normal and that the capillary network was sparse (Fig. 1, *a* and *b*). The capillary cast also revealed scattered capillary constrictions, H-shaped anastomoses, and capillary budding (Fig. 2). There were also abrupt constrictions seen in capillaries as well as in arteriolar vessels (Figs. 3 and 4). The casts also revealed various-sized vessels forming tortuous U-shaped loops that course through the placental tissue. From the smooth



Fig. 2. High magnification of a capillary cast from the placenta of a fetus with erythroblastosis fetalis. Capillary buds are shown on the right *(arrows)*, and H-shaped anastomoses of the capillaries *(arrowheads)* are shown in the center. Also seen in the central area are several constrictions of a capillary vessel. *Bar* = 10 μm.

Table I. Clinical information

Patient	*Age (yr)*	*Gravidity and parity*	*Rh titer*	*OD_{450}*	*Gestational age (wks)*	*Sex of baby*	*Apgar scores*	*Mode of delivery*	*Weight of baby*	*Fetal complications*
M. L.	29	G4P3	1:1024	0.35	30	Male	1-4-7	Cesarean section	1640	Fetal ascites Severe anemia
I. R.	25	G3P2	1:128	0.27	25	Male	3-6-8	Cesarean section	1740	Severe anemia
F. C.	22	G2P0	1:128	0.10	34	Female	3-4-6	Cesarean section	700	Intrauterine growth retardation Severe anemia
C. B.	31	G3P1	1:1024	0.34	31	Female	2-7-7	Cesarean section	1440	Severe anemia

OD_{450} = Optical density at 450 nm.

surface, it is deduced that the U-shaped vessels are veins and venules (Fig. 5, *a* and *b*). Although only four Rh-incompatible placentas were examined for this study, the vascular casts as examined by scanning electron microscopy had a reproducible appearance.

Scanning electron microscopy of whole-surface Rh-sensitized placental sections showed that large portions of the chorionic villi had occluded or collapsed capillary vessels (Fig. 6, *A*). Many of the villi appeared to be filled with fibrous tissue rather than with patent blood vessels. There were, however, some areas in which the chorionic villi had a more normal appearance (Fig. 6, *B*). In these regions, the capillaries were patent and did not have occlusions of the lumina or fibrinoid deposits. The umbilical cord was cut and processed for scanning electron microscopy. Fig. 7 is a cross section of the cord showing a dilated umbilical vein filled with the injection medium and two arteries, each with a constricted lumen.

Light microscopic observations of this pathologic tissue showed areas of histologic abnormalities as well as areas with more normal chorionic villi. A variability in microscopic appearance of chorionic villi from the same placenta occurred often. Also the villi in some regions had stromal edema with little to no space between them; in other regions, distinct intervillous spaces were seen (Fig. 8, *a* and *b*). The villi also had some areas that contained very few capillaries, giving these villi an immature appearance at term. These groups of immature villi were scattered throughout the placenta. The syncytiotrophoblast appeared normal for term, but the cytotrophoblastic cells were large and distinct.



Fig. 3. An area of loosely packed capillaries. An abrupt constriction is seen in one of the capillary vessels *(arrow)*. *Bar* = 10 μm.

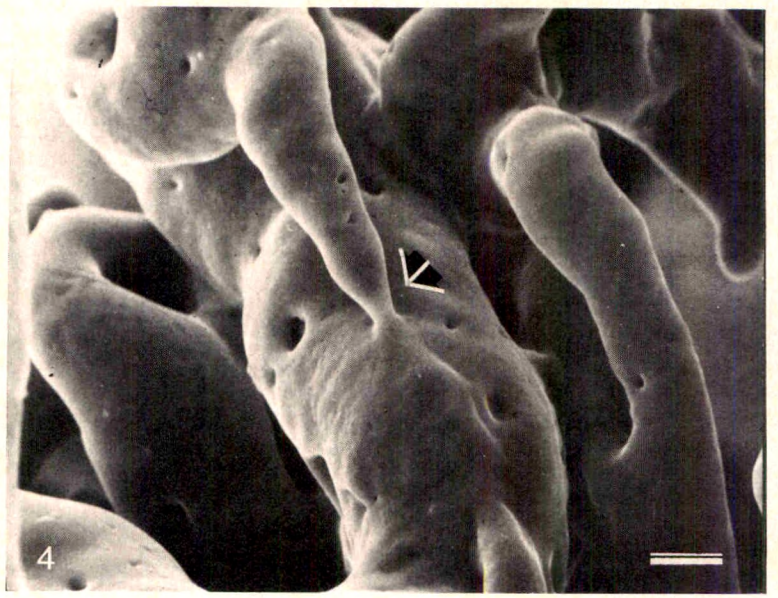


Fig. 4. Vascular cast of an arterial vessel with an abrupt constriction of one of its branches *(arrow)*. *Bar* = 10 μm.

Comment

Our scanning and light microscopic observations of Rh-sensitized placentas are in support of previous investigators' findings of variability in appearance of chorionic villi, some with edema and some with a normal appearance. Also some villi had very few capillaries and presented an immature appearance at term.[4-6, 9]

In this study we examined the fetal microcirculation of placentas in cases of severe erythroblastosis fetalis. Scanning electron microscopic examination of the latex corrosion casts provided new information on the vasculature by showing, in three dimensions, that the large vessels (arteries and veins) had less branching than those from normal placentas and that there were many areas where the capillary network was sparse. The capillary casts also revealed that there were numerous constrictions and H-shape anastomoses as well as capillary budding. This neovascularization has been observed in placental casts from small for gestational age fetuses. A similar type of capillary budding was observed by Burger et al.[10] in rat corneal capillary casts after chemical cauterization. A possible reason for the placental

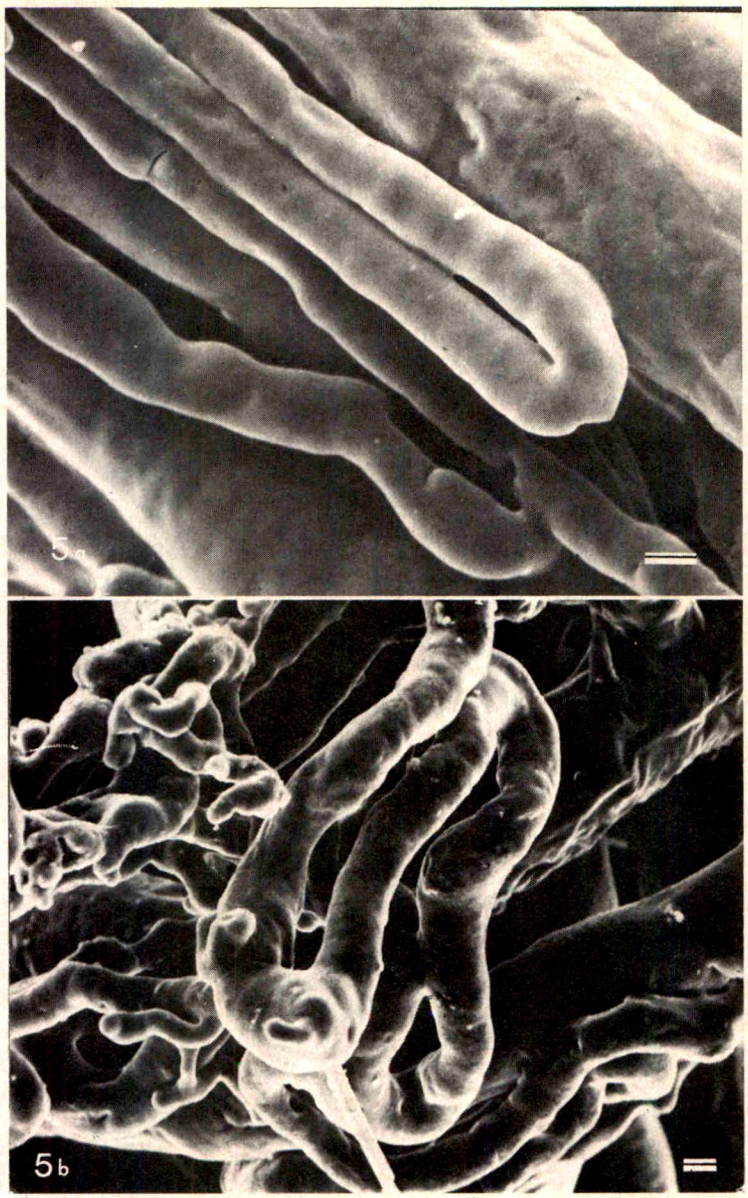


Fig. 5. *a*, Vascular cast of venule exhibiting the U-shaped appearance of one of its loops. The venules have a smooth surface. *Bar* = 10 μm. *b*, Vascular cast of a smooth-surfaced venule with three U-shape bends as it courses through the placental tissue. *Bar* = 10 μm.

capillary neovascularization is that there are insufficient capillary exchange surface areas for the developing fetus as the result of diminished villous vessels, similar to observations made in the SGA placental vasculature.[3] This reduction stimulates the capillaries to form new ones to augment the existing sparse network. The reason for a sparse capillary network in these placentas cannot be ascertained from corrosion cast examination; however, we speculate that there was some interruption in fetal-placental vasculature formation at a fairly early stage, resulting in reduction both of branching of large vessels and of density of the capillaries. The scattered arteriolar and capillary constrictions seen on the casts are probably due to areas of intracapillary erythropoiesis or to the presence of fibrinoid deposits pressing on the blood vessels, thus decreasing the vascular diameter.

Scanning electron microscopy of the vascular casts also revealed vessels of various diameters (20 to 100 μm), with distinct U-shaped turns, coursing through the placental tissue. From the smooth surfaces of these vessels, they appear to be venous structures. The U-shaped undulations of these vessels are remarkable in appearance; such structures were not observed in casts from normal human placentas or from SGA placentas. In all placentas, the injecting pressure was around 50

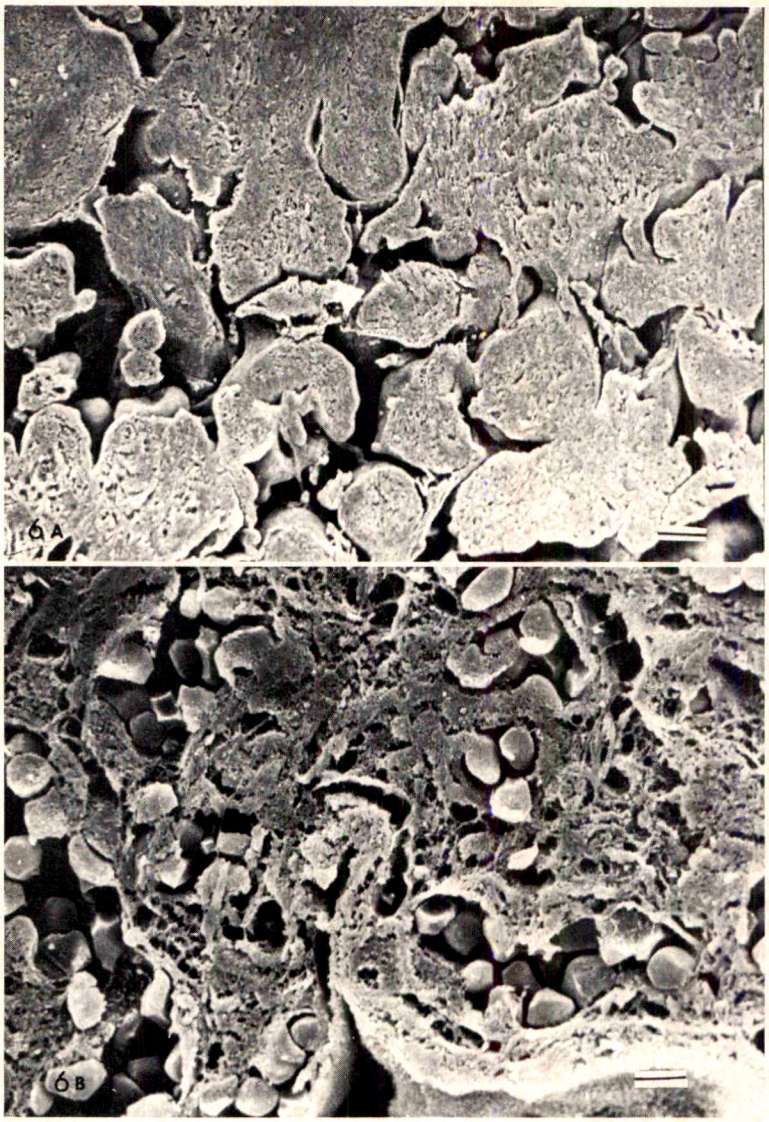


Fig. 6. *A*, A low-magnification electron micrograph of a section of the chorionic villi from the placenta of a fetus with erythroblastosis fetalis. Note that most of the capillaries are collapsed or occluded so that very few appear patent. *Bar* = 100 μm. *B*, A higher magnification of a section of the chorionic villi from the same placenta showing an area with a more normal appearance. The capillaries are patent in many areas. *Bar* = 10 μm.

mm Hg, slightly less than physiologic pressure. Therefore, the presence of U-shaped loops would indicate that the vessels existed in the living placenta under high pressure. In severe erythroblastosis fetalis, hepatosplenomegaly develops in the fetus, resulting in venous hypertension. This produces impedance in umbilical flow to the placenta. With pressure building up in the fetal-placental vessels, edema will result in the chorionic villi, with veins and venules becoming U-shaped loops under high pressure. Under this condition, the umbilical vein will become distended. This dilation was observed in the cross section of the umbilical cord from cases of severe erythroblastosis fetalis. From these scanning electron microscopic studies of the vascular morphologic features, we can better interpret the histologic findings and are also able to deduce the existing physiologic flow conditions in this abnormal placenta. Future examination of vascular casts from other types of abnormal placentas will provide additional information on fetal-placental microcirculation. Such ultrastructural data will be helpful to both placentologists and physicians.

We thank Dr. Shu Chien, Department of Physiology, for the kind use of his laboratory facilities for this study. We also thank Honor O'Sullivan and Gerard Norwich for their fine technical assistance.

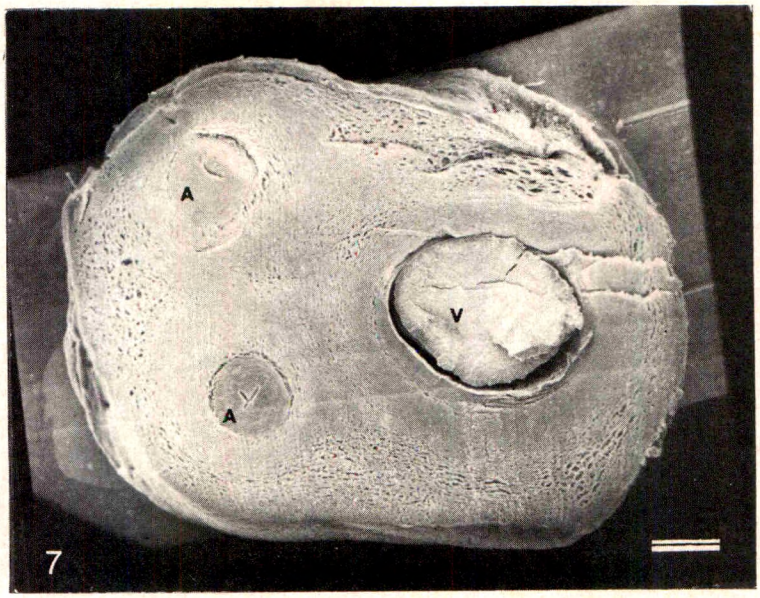


Fig. 7. A cross section of the umbilical cord from the placenta of a fetus with erythroblastosis fetalis. The umbilical arteries *(A)* are both constricted. The vein, which was filled with Cementex under physiologic pressure, is patent. *Bar* = 1 mm.

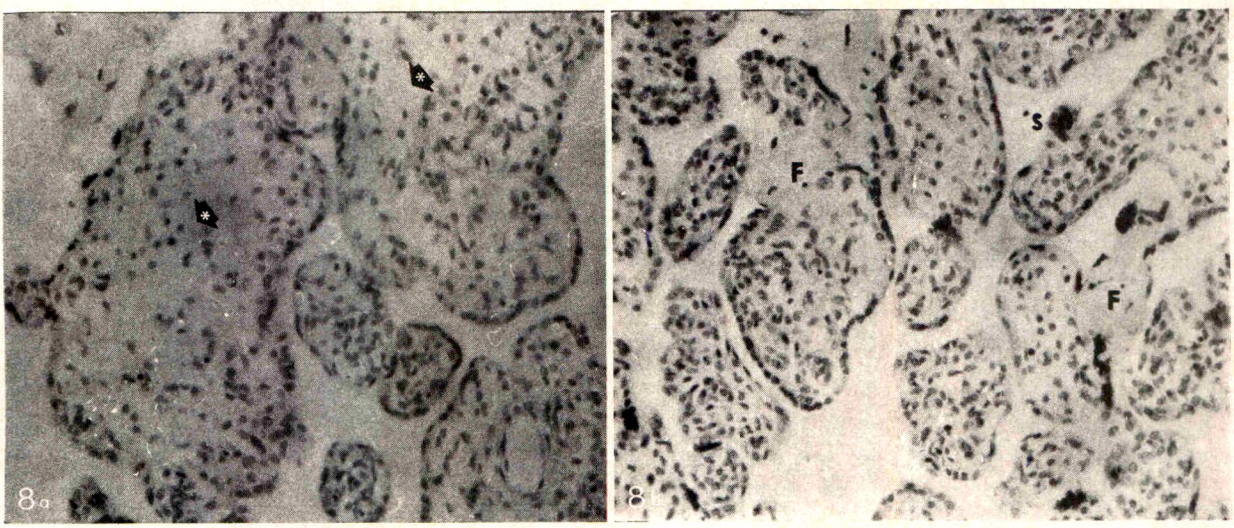


Fig. 8. *a,* Light microscopic photograph of a portion of the chorionic villi from the placenta of a fetus with erythroblastosis fetalis. Two large villi *(asterisks)* are edematous with only a tight intervillous space between them. (Hematoxylin and eosin. ×350.) *b,* Light microscopic photograph of another portion of the same placenta showing a nonedematous region with a good amount of intervillous space. Also seen are several syncytial knots *(s)* and some fibrinoid deposits *(F)*. The cytotrophoblastic layer is clearly seen on many of the villi. The villi have an immature appearance for a term placenta because many of the villi do not have a rich capillary supply. (×350.)

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Uterine metabolism of the pregnant rabbit under chronic steady-state conditions

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The study of uterine metabolism in pregnancy under chronic steady-state conditions has been confined to large mammals and, more recently, to the guinea pig. The pregnant rabbit is of interest because of its short gestation and large litter size. We developed an indirect approach involving retrograde catheterization of the uterine venous drainage, permitting measurement of both uterine metabolic quotients and uterine uptakes. Radioactive microspheres were used to measure blood flow. A large lactate and ammonia efflux from the uterus was found. In the fed state, ketogenic substrates were taken up in small amounts. However, during starvation a significant increase in ketoacid uptake was observed with a concurrent fall in acetate uptake. There was a large glucose/oxygen quotient across the uterus, but the glucose plus lactate/oxygen quotient was comparable to that found in the sheep and guinea pig (0.6 ± 0.1). It is apparent that in all three species studied under chronic steady-state conditions (sheep, guinea pig, and rabbit) there is a large glucose uptake associated with a net lactate producton, and fuels other than glucose and lactate must be used by the uterus. (Am J Obstet Gynecol 1986;154: 1146-51.)

Key words: Uterine metabolism, uterine metabolic quotients, ammonia and lactate production, glucose and lactate quotients, placental metabolism

The metabolic demands of the developing conceptus remain an important area of interest in mammalian reproductive physiology. Metabolic requirements of pregnancy have previously been described in large mammals[1] and, more recently in our laboratory, in the guinea pig.[2] Among mammals, the variations of litter size, maturation, tissue composition, and maternal caloric requirements preclude simple interspecies comparisons. Studies in conscious, unstressed sheep,[1] cow,[3] and guinea pigs[2] have described glucose as the major oxidative substrate of the uterus. A net lactate efflux from the uterus has also been described in all three species.[2-5]

There have been few studies in small mammals carried out under chronic steady-state conditions. Recently, our laboratories have reported data on the cardiac output and uterine blood flow of the pregnant rabbit under chronic steady-state conditions, a study which involved catheterization of the arterial and peripheral venous circulations.[6] We now report successful chronic catheterization of the uterine venous drainage, permitting studies of oxygen and substrate uptake by the gravid uterus under steady-state conditions.

Material and methods

Ten New Zealand White rabbits were artificially inseminated permitting precise pregnancy dating (within 1 day). During the last half of gestation, the animals were anesthetized with ketamine hydrochloride, 50 to 150 mg intramuscularly (Parke Davis & Co.), and

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Table I. Summary of maternal and fetal data

Animal No.	Gestational age (days)	Maternal weight (gm)	Whole uterus			Left horn			Uterine blood flow	
			Weight (gm)	No. of fetuses	Fetal weight (gm)	Weight (gm)	No. of fetuses	Fetal weight (gm)	Both horns (ml/min)	Left horn (ml/min)
81-45	15-16	3980	106.3	9	42.6	69.1	7	33.3	24.41	14.26
81-57	22-23	4826	246.0	10	130.2	142.9	6	80.3	37.01	21.58
81-58	22-23	3838	238.4	12	125.6	98.2	5	49.2	31.89	12.71
81-56	24-25	3870	245.9	10	147.2	125.7	5	73.0	29.04	16.15
81-49	26-27	4090	445.4	10	339.1	220.4	5	167.9	85.49	43.14
81-50	26-28	3906	367.0	10	247.5	237.4	5	172.9	32.16	23.73
81-54	27-28	3920	130.3	3	65.5	88.3	2	42.7	18.59	11.02
81-55	27-28	3921	236.0	10	131.1	114.7	5	59.3	19.75	10.89

Table II. Summary of metabolic data (arteriovenous differences, uptakes, and production)

Substrate	Arteriovenous differences (μmol/ml)	Uptake	
		μmol/min	μmol/min/kg
Glucose*	0.949 ± 0.082	18.38 ± 2.78	125.58 ± 8.49
Lactate*	−1.158 ± 0.121	−23.74 ± 4.23	−151.77 ± 11.77
β-Hydroxybutyrate	0.033 ± 0.008	0.57 ± 0.14	4.23 ± 0.91
Acetoacetate	0.016 ± 0.003	0.25 ± 0.08	2.60 ± 0.38
Acetate†	0.084 ± 0.020	1.85 ± 0.40	11.78 ± 2.50
Ammonia*	−0.069 ± 0.007	−1.28 ± 0.13	−9.15 ± 1.06
Oxygen*	3.890 ± 0.214	75.23 ± 8.67	532.58 ± 38.83

Uterine uptakes calculated as product of flow to uterine horn where venous drainage was catheterized times the arteriovenous differences.

*$p < 0.001$.

†$p < 0.01$.

xylazine hydrochloride, 25 to 50 mg intramuscular (Bayes). Polyvinyl catheters were placed in the left ventricle (0.34 mm inner diameter) and distal aorta (0.23 mm inner diameter). A closed-end nylon catheter (0.34 mm outer diameter and 0.23 mm inner diameter) was placed in the left uterine vein by a retrograde technique. The retrograde catheterization of the uterine vein was carried out as follows. The catheter was inserted into the femoral vein and advanced to the junction of the common iliacs. Under direct visualization the inferior vena cava was occluded transiently so that the catheter would advance up the uterine vein. The position of the catheter tip was confirmed by direct palpation. By this approach, the uterus need not be manipulated at all and the catheter can be positioned to sample drainage from an entire uterine horn.

All catheters were secured with sutures and surgical adhesive (Eastman 910) tunneled subcutaneously to an area between the scapulas and secured in a fixed plastic cap as previously described.[7] The catheters were maintained patent by flushing daily with a heparin-saline solution (200 units/ml). The animals were given food and water as needed and postoperative food intake was measured. All animals were studied on two consecutive days after a 3-day recovery period.

Paired arterial and venous samples were drawn simultaneously for oxygen, glucose, lactate, acetate, β-hydroxybutyrate, acetoacetate, and ammonia determinations. Four sets of such samples were obtained during approximately 1 hour, and the mean arteriovenous difference for each substrate was used as the value for that day of study. After completion of sampling on the second day of study, all animals underwent injection with radioactive "carbonized" microspheres (tin 113, 24 μ in diameter) with the use of the integrated withdrawal technique previously described.[8] The animals were put to death and the uterus of each dissected to determine fetal, placental, and uterine weights. Ovaries, kidneys, and adrenals were dissected to verify paired organ flows. The calculations for uterine blood flows were done by the reference sample method.[8]

Two groups of animals were studied, those with dietary intakes of >45 gm/day (previously found to be the lower limit of normal food intake)[9] and a second group of starved animals with dietary intake <20 gm/day.

Chemical methods. All metabolic uptakes for oxygen and substrates across the uterine horn were calculated by application of the Fick principle. Oxygen content was measured with an OSM_2 hemoximeter (Radiometer). The hemoximeter has the advantage of giving highly reproducible measurements of both hemoglobin (in millimoles per liter) and oxygen saturation, from

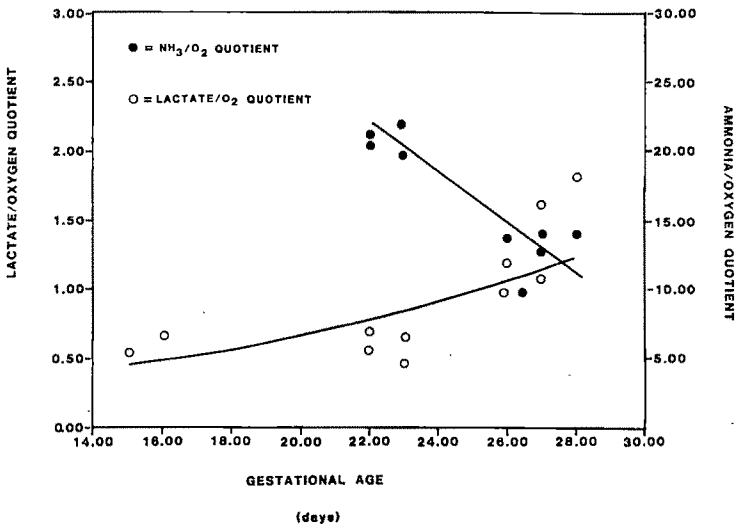


Fig. 1. Gestational changes in lactate/oxygen and ammonia/oxygen quotients across the uterus.

which the oxygen content is calculated. Glucose, lactate, acetate, acetoacetate, and β-hydroxybutyrate levels were measured with standard enzymatic methods.[10] The ammonia content was determined according to the ion-exchange method of Kurahasi et al.[11] The samples were measured in triplicate with a coefficient of variation of 4%.

The average arteriovenous concentration differences of the four sets of measurements were calculated by taking into account each nutrient used to ascertain the metabolic quotients for that day's study with the formula:

$$\frac{\text{Average arteriovenous difference of substrate (mmol)} \times k}{\text{Average arteriovenous difference of oxygen (mmol)}}$$

where k is the number of oxygen molecules required for the complete oxidative combustion of one molecule of the substrate. This number varies from two for acetate to six for glucose. The mean of the four arteriovenous differences was also used to calculate the coefficient of extraction with the following formula:

$$\frac{\text{Arteriovenous} \times 100\ (\%)}{\text{Arterial concentration}}$$

Statistical methods used were either paired or unpaired *t* tests, as designated. Regression lines were calculated by the least-squares method. All values are expressed as mean ± SEM unless otherwise noted. The significance of correlation coefficients was calculated by use of hypothesis testing.

Results

Table I presents a summary of maternal weights, gestational age, fetal and placental weights, and uterine blood flows for the entire uterus as well as for the individual uterine horn studied. The studies covered a gestational age range of 15 to 28 days.

Table II presents a summary of all metabolic data including mean arteriovenous differences and uterine uptakes or production of substrates in the horn studied. The mean arteriovenous glucose difference was 0.95 ± 0.82 μmol/ml, the oxygen difference was 3.89 ± 0.21 μmol/ml, and the lactate arteriovenous differences was −1.15 ± 0.12 μmol/ml. Small positive arteriovenous differences for ketoacids and acetate were found but did not approach statistical significance.

Expressed per kilogram of tissue (uterus plus placenta plus fetus), the rabbit uterus and its contents consumed 125.58 ± 8.49 μmol/min of glucose, 532.58 ± 38.83 μmol/min of oxygen, and 11.78 ± 2.50 μmol/min of acetate as well as small amounts of ketoacids. There was a large efflux of lactate (−151.77 ± 11.77 μmol/min) and of ammonia (9.15 ± 1.06 μmol/min) from the uterus.

Table III presents a summary of the metabolic quotients. The glucose/oxygen quotient was 1.46 ± 0.11, and the lactate/oxygen quotient was −0.91 ± 0.09. The glucose plus lactate/oxygen quotient was 0.55 ± 0.064. The ketoacid/oxygen quotients were 0.04 ± 0.01 for β-hydroxybutyrate and 0.02 ± 0.004 for acetoacetate. Acetate/oxygen quotient was 0.04 ± 0.009. The sum of all substrate quotients was 0.65 ± 0.015.

Since ammonia and lactate are both small molecules, which we believe are produced within the placenta, it was of interest to compare the quotients at different stages of gestation. Fig. 1 illustrates the fact that these quotients do not show a similar pattern of change. The ammonia/oxygen quotient falls while the lactate/oxygen quotient rises with increasing gestational age.

Table IV compares two groups of animals, one con-

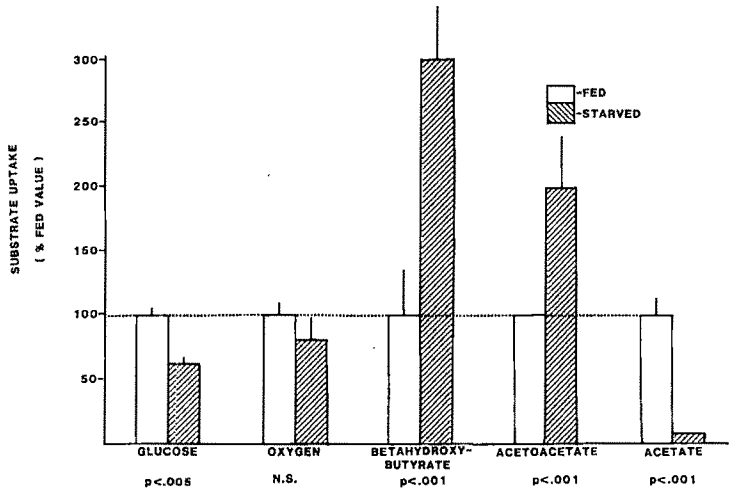


Fig. 2. The uterine uptakes of substrates by the left uterine horn in the fed and starved states expressed as a percentage of the fed state.

Table III. Metabolic quotients across the left uterine horn

	Quotient
Glucose/oxygen	1.46 ± 0.11*
Lactate/oxygen	−0.91 ± 0.09*
β-Hydroxybutyrate/oxygen	0.04 ± 0.01†
Acetoacetate/oxygen	0.02 ± 0.004*
Acetate/oxygen	0.04 ± 0.009*
Total	0.65 ± 0.015

*$p < 0.001$.
†$p < 0.005$.

Table IV. Gestational age, fetal number, and uterine weight of starved versus fed animals

	Fed (n = 11)	*Starved (n = 4)*
Food intake/day (gm)	127.6 ± 17.19	3.25 ± 2.25
Fetal age (days)	22.9 ± 2.11	25.5 ± 1.0
No. of fetuses	5.6 ± 0.4	5.0 ± 0.0
Total weight of left uterine horn (gm)	153.58 ± 33.0	120.20 ± 5.46

Values are mean ± SEM.

suming a normal quantity of food and a subgroup of starved animals (animals offered food but with decreased dietary intake). Previous observations by Gilbert et al.[9] defined an adequate dietary intake as a minimum of 45 gm/day. The gestational age and litter size were similar in the two groups. The total uterine weight was slightly decreased in the starved group (153.58 ± 33 gm in fed animals versus 120.20 ± 5.46 gm in starved animals), although this difference is not statistically significant.

Fig. 2 presents a comparison of uptakes per kilogram of left uterine horn (mean ± SEM) with uptakes in starved animals presented as a percentage of the uptakes in fed animals. The most dramatic change was in acetate uptake, which decreased 98%. Conversely, β-hydroxybutyrate and acetoacetate uptakes increased 300% and 200%, respectively. Fig. 3 demonstrates that there was an inverse relationship between uterine ketoacid uptake and daily food intake.

Fig. 4 presents a comparison of the production rates for lactate and ammonia in starved animals expressed as a percentage of the fed state across the left uterine horn. A significant 25% decrease in lactate production was found. The decrease in ammonia production was not significant.

Comment

This study presents data describing the metabolism of the rabbit uterus under chronic steady-state conditions. It should be emphasized that the surgical approach we have utilized for these studies differs from that of Gilbert et al.,[12] who recently reported metabolic data for the rabbit uterus. In the latter study, venous blood from a single fetoplacental unit and the surrounding myometrial tissue was sampled. Thus the metabolic quotients in that study reflected the metabolism of that unit. In our approach the retrograde catheterization can be achieved without any direct manipulation of the uterus or its contents and the catheter tip can be directly localized in the main venous drainage of the horn. However, it is encouraging that the two different approaches have yielded very similar estimates of glucose uptake and lactate production by the pregnant rabbit uterus, 127.5 versus 125.6 μmol/L glucose/min/kg in the study of Gilbert et al.[12] versus the present study and 122 versus 151.8 μmol/L lactate/min/

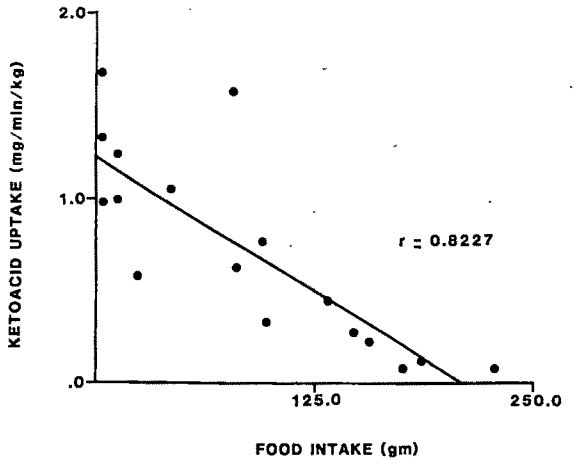


Fig. 3. Maternal food intake (grams) versus uterine ketoacid uptake (milligrams per minute per kilogram).

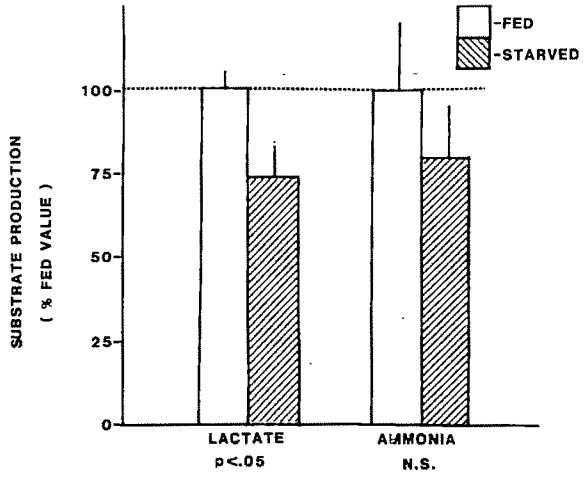


Fig. 4. Comparison of lactate and ammonia efflux from the left uterine horn in fed and starved states.

Table V. Comparison of chronic metabolic studies of three species in our laboratory

Species	*Gestation (%)*	*Glucose/oxygen quotient*	*Lactate/oxygen quotient*	*(Glucose + lactate)/ oxygen quotient*	*Coefficient of extraction (%)*	
					Glucose	*Oxygen*
Rabbit	70-95	1.5 ± 0.1	−0.9 ± 0.1	0.6 ± 0.1	19.8 ± 1.7	53.0 ± 3.3
Guinea pig	60-75	1.2 ± 0.1	−0.5 ± 0.1	0.7 ± 0.1	11.9 ± 1.3	39.0 ± 2.8
Sheep	80-95	0.8 ± 0.1	−0.1 ± 0.0	0.7 ± 0.1	9.0 ± 0.6	24.2 ± 1.1

Values are mean ± SEM.

kg, respectively. Our laboratory has previously presented the results of studies on the metabolism of sheep[1, 4, 5] and guinea pig uteri.[2] Coupled with the similar studies of Comline and Silver[3, 13] on horse and cow uteri, five mammalian species have now been studied under comparable steady-state conditions.

In all five mammalian species studied thus far, uterine metabolism has been characterized by a very large glucose/oxygen quotient across the uterine circulation, reflecting the high rate of lactate production within the placenta. Table V presents the glucose plus lactate/oxygen quotients for the three species in which complete data are available. The quotients are significantly <1.0 and, in fact, are strikingly similar among the three species, which otherwise differ in a variety of important ways. In contrast, the coefficients of extraction for glucose and oxygen are quite different among these species, reflecting differences in perfusion of the uterus relative to its metabolic rate with the three species.

Another characteristic of uterine metabolism that is true in the rabbit, as in other species, is the efflux of ammonia. In the sheep it has been possible to demonstrate that the uteroplacental tissues are the source of the ammonia, not the fetal tissues. This was achieved by demonstrating that there was a significant entry of ammonia into both the umbilical circulation and the uterine circulation.[14] Since the umbilical circulation or fetal side of the placenta cannot be studied chronically in the rabbit, it is possible to demonstrate only that ammonia is produced within the uterus as a whole and represents a significant efflux of nitrogen from the pregnant uterus.

During gestation the uterine oxygen quotients for ammonia and lactate do not show a similar pattern of change. This observation suggests that these changes are not simply a reflection of changes in the proportion of placental tissue to total uterine contents, because, in the latter case, both quotients would show a similar pattern of change. Presumably, these changes must reflect a basic change in metabolism of the placenta with an increasing lactate production and decreasing ammonia production relative to the metabolic rate of the uterus.

In summary, 10 pregnant rabbits were chronically catheterized during the last half of pregnancy. Catheters were placed in the left ventricle and the distal aorta and into the main venous drainage from one pregnant uterine horn. Metabolic quotients across the uterus were measured for glucose, lactate, ketoacids, acetate and ammonia. In addition, uterine blood flow was measured by the integrated reference sample technique and microspheres 25 μm in diameter. A large lactate and ammonia efflux from the uterus was found. The glucose plus lactate/oxygen quotient was 0.6 ± 0.1.

There was a small but significant uptake of ketoacids and acetate by the uterus with the animals in the fed state. Ketoacid uptake increased and glucose and acetate uptake markedly decreased when maternal food intake was reduced. These uterine metabolic data collected under chronic steady-state conditions were compared with data collected under similar conditions for the sheep and guinea pig. While the coefficients of extraction differ among the species, the glucose plus lactate/oxygen quotients are remarkably similar for the uteri of all three species.

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Maternal ovine placental vascular responses to adenosine

Megan Landauer, M.D., Terrance M. Phernetton, B.S., and John H. G. Rankin, Ph.D.
Madison, Wisconsin

We have examined the effect of adenosine given via retrograde uterine artery catheter at 25 mg/min to nine chronically catheterized near-term sheep pretreated with angiotensin II, 5 μg/min, via the jugular vein. Blood flows were measured by the microsphere technique. Flows were measured at rest, after 10 minutes of angiotensin II infusion, and after 1.5 minute of adenosine infusion. Blood pressure increased with angiotensin II infusion from 101 ± 2.63 to 130 ± 4.33 mm Hg ($p < 0.05$) and then remained unchanged with local adenosine infusion at 129 ± 4.33 mm Hg (NS). Organ resistances were:

Tissue	*Control*	*Angiotensin II*	*Adenosine*
Uterine resistance/gm	299 ± 0.04	767 ± 83 ($p < 0.05$)	355 ± 37.7 ($p < 0.05$)
Cotyledonary resistance/gm	37.9 ± 4.83	48.1 ± 4.93 ($p < 0.05$)	50.10 ± 5.27 ($p < 0.05$)

The experimental uterine tissue resistance increased with angiotensin II and showed vasodilation in response to adenosine. The cotyledonary resistance increased with angiotensin II infusion but showed no change in response to adenosine. The maternal-placental response was anomalous. Angiotensin II–induced maternal-placental vasoconstriction cannot be opposed by the exogenous administration of adenosine. (Am J Obstet Gynecol 1986;154:1152-5.)

Key words: Fetus, adenosine, angiotensin II, placenta, microspheres, blood flow

The factors that regulate maternal-placental blood flow are not fully understood. For example, we originally demonstrated that prostacyclin, which is a potent vasodilator,[1-3] is not effective in dilating the placental vasculature in the ewe. The systemic administration of prostacyclin via the jugular vein to a resting, unanesthetized sheep did not cause placental vasodilation.[3] In an attempt to avoid the systemic responses to prostacyclin we then applied the substance locally via retrograde uterine arterial catheter and again observed no dilation of the placental vascular bed.[1] In a third series prostacyclin was administered in the near-term sheep in association with angiotensin II in order to provide a vascular bed that had some vasodilatory reserve, and yet we still observed no vasodilation with prostacyclin.[2] An explanation for this anomalous result would be either that the sheep placenta was unresponsive to prostacyclin specifically or that this vascular bed was not responsive to other vasodilators. We therefore performed a series of experiments with adenosine used as the vasodilator.

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Material and methods

Nine chronically catheterized near-term mixed breed ewes were used. Maternal drug administration was by jugular vein for angiotensin II and retrograde uterine artery catheter for adenosine. The uterine tissue not served by the cannulated artery was used for comparison as were the renal tissues.

Surgical procedure. All procedures were performed with the animal anesthetized by intramuscular ketamine (10 mg) and intravenous ketamine (10 mg/ml) and supplemental Nembutal as necessary. Animals were fasted for 48 hours and water was withheld for 24 hours before operation. The maternal jugular vein, left ventricle, and femoral arteries were catheterized. A ventral midline incision was made and the uterine vasculature exposed. The middle uterine artery was dissected free and a catheter passed in retrograde fashion to the branch point of the artery. The smaller of the two branches was chosen for catheterization. The peritoneum was oversewn and the catheter anchored to the uterus. All catheters were brought out via a subcutaneous tunnel and placed in a pouch on the flank. The abdominal incision was closed. The ewes were given an intramuscular injection of penicillin immediately after operation. Details of the surgical methods have been published previously.[1, 4, 5]

Flow measurements. For measurement of organ flows microspheres were injected in the left ventricle while blood was withdrawn from the femoral artery at 4.2 ml/min during and for 2 minutes after the micro-

Table I. Blood pressures, blood flows, and resistances of uterine and cotyledonary tissue downstream from retrograde catheter and of renal tissue

	Control	*Angiotensin II*	*Angiotensin II plus adenosine*
Blood pressure (mm Hg)	101 ± 2.63	130 ± 4.33*	129 ± 4.33
Renal resistance/gm	42.89 ± 1.69	92.64 ± 3.66*	137.59 ± 32.89
Uterine flow/gm	0.40 ± 0.07	0.19 ± 0.02*	0.40 ± 0.05*
Uterine resistance/gm	299 ± 40.0	767 ± 83.0*	355 ± 37.7*
Cotyledonary flow/gm	3.27 ± 0.67	3.0 ± 0.33	2.77 ± 2.67
Cotyledonary resistance/gm	37.9 ± 4.83	48.1 ± 4.93*	50.1 ± 5.27

Resistance is expressed as millimeters of mercury per milliliter per minute per gram. Flows are expressed as flow per gram of tissue. Values are mean ± SE.

*$p < 0.05$ compared with preceding value.

sphere bolus injecton. Spheres were 15 μm in diameter and labelled with either cerium 141, strontium 85, iodine 125 or scandium 46. The spheres were suspended in 10% dextran in normal saline solution and consisted of 15 μCi of radioactivity in 0.4 ml. Each injection consisted of approximately 2.5×10^6 microspheres.

Experimental procedures. Experiments were performed 4 days after operation. The ewe was allowed to acclimate to a stanchion for 2 hours on the morning of the experiment. Blood pressure and pulse were recorded continuously by a transducer placed at the level of the scapulohumeral joint and were obtained via a femoral artery catheter.

Blood flow was measured before infusions were begun for control flow determinations. Angiotensin II was infused at 5 μg/min into the jugular vein for 10 minutes and a repeat blood flow measurement was performed. Adenosine was then given via retrograde uterine artery catheter at 25 mg/min via pump, in addition to the continuing angiotensin II infusion. A final microsphere bolus was given after 1.5 minutes of angiotensin II plus adenosine administration and the final test blood flows were obtained. The ewe was put to death at the end of the experiment while green dye was infused into the retrograde catheter. We have used this technique extensively and the dye is distributed to those areas served by the cannulated artery. The dye is taken up by both uterus and cotyledons and serves to map the experimental tissues. Removal of the uterus and its contents and the kidneys was performed immediately by laparotomy. Experimental (green) tissues were separated from control (nondyed) tissues.

Measurement and tissue preparation. Tissues were processed immediately. Cotyledons were analyzed individually and sample aliquots taken of homogenized renal and uterine tissues. All samples were assayed with a Nuclear Chicago Model 1185, three-channel gamma counter. The height of the tissue in the bottle was <1 cm above the bottom of the sampling vial. Three isotopes were used and the sample stream was preceded by three standard vials with known numbers of spheres of each isotope embedded in wax, followed by the femoral artery withdrawal blood samples. The spillover of each isotope into the other channels was calculated from the standard vials. The number of spheres in each sample was determined after calculation of spillover, and blood flow per organ was calculated from the number of spheres per organ relative to the number of spheres in the withdrawal blood samples by use of previously described equations.[4] Results are expressed as mean ± SEM. Statistical analysis of control, angiotensin II, and angiotensin II plus adenosine flows and resistances was done by analysis of variance with a $p < 0.05$ significance level.

Results

The results are given in Table I. It can be seen that the maternal blood pressure increased significantly with angiotensin II infusion as would be expected. Blood pressure remained elevated during the local infusion of adenosine, which indicated that no major systemic responses to adenosine were present at the time the observation was made. The flow per gram of the experimental uterine tissue could be seen to decrease significantly during the angiotensin II infusion and then to increase significantly during the administration of adenosine. The resistance of this vascular bed increased with angiotensin II and decreased with adenosine. This is the classical response that would have been predicted from the established literature. The renal vascular bed showed vasoconstriction in response to angiotensin II and did not dilate in response to the local administration of adenosine to the uteroplacental vascular beds. This again supports the contention that at the time the third measurement was made there was little or no recirculation occurring. The nonexperimental uterine and cotyledonary tissues behaved as did the renal vascular bed, showing constriction in response to angiotensin II and no vasodilation in response to adenosine.

It is the response of the placental vascular bed that

is most important. It can be seen from the data in Table I that the blood flow to the placenta did not change during the infusion of angiotensin II. This is to be expected from the literature in that the combination of vasoconstriction and elevated blood pressure served to maintain the blood flow.[6] However, during the local administration of adenosine there was no increase in placental blood flow. This is the anomalous response. It can be seen that the vascular resistance increased with angiotensin II and thereafter did not change even though that tissue was subjected to relatively massive doses of adenosine and had some vasodilatory reserve. The local infusion of adenosine could be shown to reverse the angiotensin II–provoked uterine nonplacental vasoconstriction in that tissue served by the retrograde catheter. The cotyledonary vasculature was not responsive to adenosine and maintained an increased resistance associated with the systemic infusion of angiotensin II.

Comment

Blood flow to the female reproductive organs is responsive to the changes in hormone activity during menstrual or estrous cycling and during the unique hormone changes of pregnancy.

Both Resnik et al.[7] and Still and Greiss[8] have shown that uterine blood flow is increased by adenosine in the nonpregnant sheep. Fleet and Heap[9] have demonstrated a dose-dependent increase in uterine blood flow in response to adenosine infusion into the uterine artery via a surgically created femoral–uterine arterial shunt. In their series, nonpregnant animals responded more dramatically than did ewes on day 15 of a fertile mating. Fox and Kurpis[10] postulated the existence of a low-affinity stimulatory receptor identified in the microsomal fraction of fresh human placental homogenate and hypothesized that adenosine may stimulate blood flow locally in the placenta.

It has been stressed that adenosine is very likely a mediator of coronary blood flow and is released rapidly under hypoxic conditions with resultant dramatic increases in coronary blood flow.[11]

In our previous experiments we have demonstrated that prostacyclin, a potent uterine vasodilator in both nonpregnant and pregnant sheep, is not effective in dilating the placental vasculature. When given as an injection systemically to the near-term ewe, prostacyclin caused hypotension and vasodilation in the maternal uterus, kidneys, heart, and brain but caused vasoconstriction in the placental circulation with a 43% decrease in blood flow to the cotyledonary tissue.[3] When given as a local infusion via the uterine artery, prostacyclin caused an increase in local uterine blood flow but no change in local cotyledonary blood flow.[1] In a final series, prostacyclin was given systemically after pretreatment with systemic angiotensin II.[2] In this model, we ensured that the vasculature was constricted before prostacyclin administration. In this manner we established that prostacyclin did reverse the pharmacologic constriction of the renal and uterine vasculature but did not dilate the constricted placental vascular bed. We hypothesized that adenosine, acting via a different receptor system, might be capable of dilating a placenta previously contracted by angiotensin II.

In our experiments, the uterus responded in the predicted manner. We are confident that the local application of adenosine was effective in that there was no change in systemic blood pressure or renal resistance during the short adenosine infusion. The cotyledons that were "downstream" from the retrograde catheter should have received a concentration of adenosine similar to that traversing the adjacent myometrium. We conclude that placental vascular responses are modulated by mechanisms that are significantly different from those controlling the nonplacental uterine vascular bed. Furthermore, the prior constriction of the placenta with angiotensin II guaranteed a vasodilatory reserve in these vessels so that they could have dilated had adenosine been an effective mediator. Neither prostaglandin I_2 nor adenosine causes placental dilation in the near-term ewe although they both cause vasodilation of the adjacent uterine vasculature. Prostaglandin I_2 and adenosine should act by stimulating different sets of endothelial receptors, indicating that the placental response to vasodilators in general is anomalous.

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Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit

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The effects of pharmacologic doses of gonadotropin-releasing hormone, danazol, and indomethacin on the clinical and endocrinologic features of the ovarian hyperstimulation syndrome were studied in the rabbit. The ovarian hyperstimulation syndrome was induced with Pergonal (75 IU of follicle-stimulating hormone and 75 IU of luteinizing hormone) and a follicle-stimulating hormone–dominant gonadotropin preparation (85 IU of follicle-stimulating hormone and 53 IU of luteinizing hormone). None of the three agents tested were effective in suppressing the ovarian enlargement and ascites formation in these animals. Ascites developed despite quite significant variations in plasma and intraovarian sex steroid hormone and intraovarian prostaglandin F levels induced by danazol and indomethacin. Ascites develops in hyperstimulated women in association with both follicular and luteal hyperstimulation. In contrast, the ascites response in the hyperstimulated rabbit develops in the presence of follicular hyperstimulation alone without a significant degree of luteal hyperstimulation. (Am J Obstet Gynecol 1986;154:1155-60.)

Key words: Ovarian hyperstimulation syndrome, danazol, indomethacin, gonadotropin-releasing hormone

The ovarian hyperstimulation syndrome is a well-recognized and potentially life-threatening risk associated with ovulation induction therapy, particularly with exogenous gonadotropins.[1,2] The full-blown clinical syndrome occurs after ovulation and, if conception occurs, the process tends to be more prolonged and severe. With improved monitoring of the response to treatment, the incidence of this iatrogenic complication has been reduced but not eliminated.[1] This is, in part, a function of the narrow therapeutic index and the sometimes unpredictable patient sensitivity to such therapy. The incidence of mild, moderate, and severe ovarian hyperstimulation syndrome has been reported to vary from 8% to 23%, <1% to 7%, and <1% to 10%, respectively.[1] As ovulation induction therapy with gonadotropins becomes more widely available and the indications for its use expand (for instance, ovulation induction for in vitro fertilization), this complication may assume greater clinical importance.

The fundamental pathophysiology of ovarian hyperstimulation syndrome, particularly the third-space fluid sequestration, is not understood and specific therapeutic measures that might arrest or reverse this are not available. The purpose of this study was to determine if the pathologic manifestations of ovarian hyperstimulation syndrome could be influenced by the administration of pharmacologic doses of three compounds: gonadotropin-releasing hormone (GnRH), danazol, and indomethacin. Although each of these three agents is known to interfere with ovulation and/or ovarian steroidogenesis, these effects do not always

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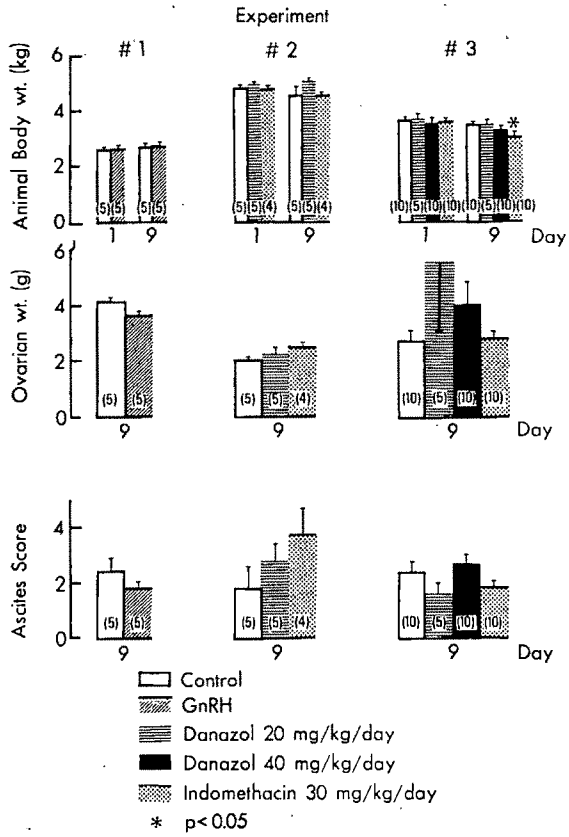


Fig. 1. Physical characteristics (body weights on days 1 and 9, ovarian weights and ascites scores on day 9) for control and test animals in experiments 1, 2, and 3. The numbers in parentheses refer to the number of animals in each group. Values are expressed as mean ± SEM. Comparisons and statistical analyses were drawn only between the treatment groups and controls within each experiment.

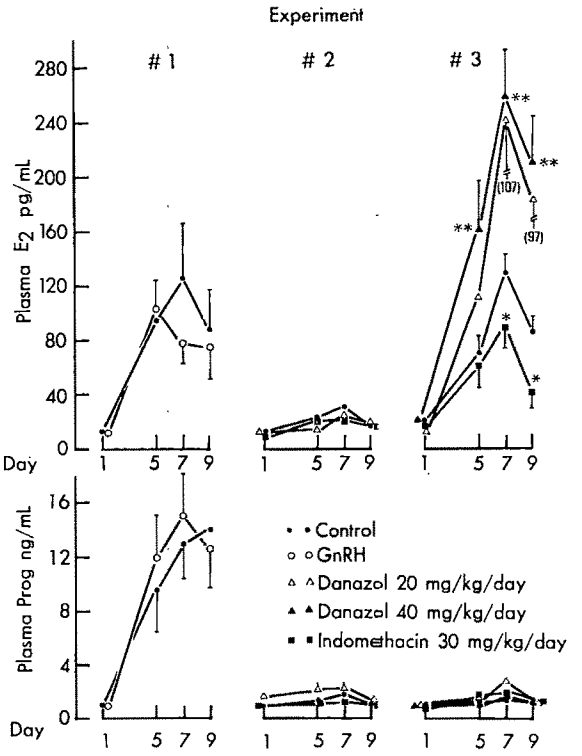


Fig. 2. Serial plasma levels of estradiol (Ξ_2) and progesterone *(Prog)* for control and test animals in experiments 1, 2, and 3 during the 9 days of the experiment. Values are expressed as mean ± SEM. Comparisons and statistical analyses were drawn only between the treatment groups and controls within each experiment. * = $p < 0.05$. ** = $p < 0.01$.

apply to the normal unstimulated rabbit ovary.[3-10] Pharmacologic doses of both GnRH and its agonist analogues have well-documented effects on ovarian steroidogenesis, which are demonstrable in both intact and hypophysectomized animals, supporting a direct gonadal action.[3-6] Danazol, an isoxazole derivative of the synthetic steroid 17α-ethinyl testosterone (ethisterone), competitively inhibits multiple enzyme systems involved in steroidogenesis in both the ovary and adrenal.[7,8] Indomethacin is a well-known and potent prostaglandin synthetase inhibitor that can prevent follicular rupture and ovum release.[9] Furthermore, in the rabbit model of ovarian hyperstimulation syndrome this compound may inhibit ascites formation.[10]

Material and methods

Fifty-nine virgin New Zealand White rabbits were used in three experiments. Each experiment had its own control group.

Experiment No. 1. Ten rabbits weighing between 2.5 and 3.1 kg were used. Both control (n = 5) and test animals (n = 5) received one ampule of Pergonal [75 IU of follicle-stimulating hormone (FSH) and 75 IU of luteinizing hormone (LH)] intramuscularly daily for 7 days. Test animals also received GnRH (Factrel), 440 μg/kg intramuscularly twice daily for 8 days.

Experiment No. 2. Fourteen animals (3.3 to 5.4 kg) were divided into three groups. One ampule of an FSH-dominant gonadotropin preparation (85 IU of FSH and 53 IU of LH) was given intramuscularly once daily for 7 days to both control (n = 5) and test animals. In addition, test animals received either danazol, 20 mg/kg subcutaneously (n = 5), or indomethacin, 30 mg/kg subcutaneously (n = 4) once daily for 8 days. Danazol was suspended in propylene glycol and indomethacin was suspended in sesame seed oil.

Experiment No. 3. Thirty-five animals (2.8 to 4.7 kg) were divided into four groups. Control (n = 10) and all test animals (n = 25) received one ampule of Pergonal intramuscularly once daily for 7 days. Five test animals received 20 mg/kg of danazol subcutaneously once daily for 8 days; 10 animals received 20 mg/kg of danazol subcutaneously twice daily for 8 days (total dosage of 40 mg/kg per day); and 10 animals received indomethacin, 30 mg/kg subcutaneously once daily for 8 days.

In all three experiments, animals were weighed on the first day and the last day (day 9). Serial blood samples from a marginal ear vein were drawn on days 1, 5, 7, and 9, just before administration of medications. Animals were killed by an overdose of pentobarbitol sodium (Euthansol) on the ninth day and necropsies were performed immediately. Ascites formation was assessed and assigned a score.[11] After excision, both ovaries were immediately washed in cold saline solution, blotted dry, weighed, then quickly placed in ice-cold absolute ethanol and homogenized. In experiment 3, representative sections of ovaries were excised, placed in formalin solution, and submitted for routine histologic evaluation in five controls, five animals receiving danazol, 40 mg/daily, and five animals receiving indomethacin.

Plasma and ovarian homogenate content of 17β-estradiol and progesterone and ovarian homogenate prostaglandin F were assayed by specific radioimmunoassays.[11]

Statistical analyses. Statistical analyses were performed by the Mann-Whitney test, linear correlation analysis, and the χ^2 test where appropriate. Data were compared between the treatment and control groups within each experiment. All data are presented as mean ± SEM.

Results

Experiment No. 1. Mean body weights for control and GnRH-treated animals were comparable and did not change during the course of the experiment. No differences were observed in mean ovarian weights, mean ascites score, serial plasma and ovarian 17β-estradiol, and progesterone and ovarian homogenate prostaglandin F (Figs. 1, 2, and 3).

Experiment No. 2. Mean body weights of control and test animals were similar and did not change during the course of the experiment. Compared with controls, danazol- and indomethacin-treated animals showed no appreciable differences in ovarian weights or in plasma and ovarian homogenate sex steroid concentrations (Figs. 1, 2, and 3). The ovarian homogenate prostaglandin F content of indomethacin-treated animals was significantly lower than in both control and danazol-treated animals (13.82 ± 4.41 versus 79.8 ± 30.58 and 71.18 ± 18 pg/mg of protein, respectively; $p < 0.05$; Fig. 3). One of five controls, four of five danazol-treated animals, and three of four indomethacin-treated animals developed ascites with scores ranging between 1 and 5, and the mean ascites scores are shown in Fig. 1.

Experiment No. 3

Controls (n = 10). Mean animal weights were comparable at the beginning and end of the experiment (3.71 ± 0.09 and 3.56 ± 0.08 kg). The mean ovarian weight was 2.78 ± 0.33 gm (Fig. 1; range 1.41 to 4.97 gm). All ovaries showed multiple cystic and hemorrhagic follicles over the external surfaces with few if any corpora lutea. Histologic assessment confirmed the presence of hemorrhagic follicles, cystic follicles, and minimal corpora lutea formation. Interstitial tissue generally exhibited little luteinization. Seven of the 10 controls developed ascites; the mean ascites score for this group was 2.4 ± 0.34 (Fig. 1). Plasma 17β-estradiol levels were 20.2 ± 4.5, 70.6 ± 13.76, 133.2 ± 13.2, and 85.7 ± 13.1 pg/ml on days 1, 5, 7, and 9, respectively (Fig. 2). Plasma progesterone levels did not exceed 5 ng/ml at any time. Ovarian homogenate 17β-estradiol and progesterone levels were 238.4 ± 43.6 pg/mg of protein and 3.6 ng/mg of protein, respectively. The homogenate content of prostaglandin F was 17.59 ± 2.17 pg/mg of protein (Fig. 3).

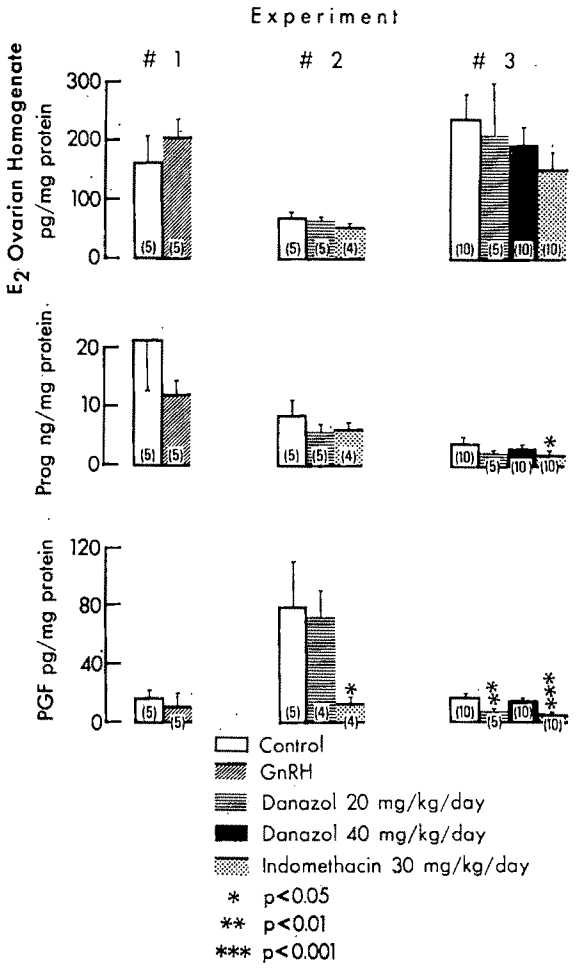


Fig. 3. Ovarian homogenate content of estradiol *(E_2)*, progesterone *(Prog)*, and prostaglandin F *(PGF)* expressed per milligram of protein in control and test animals in experiments 1, 2, and 3. Values are expressed as mean ± SEM. Comparisons and statistical analyses were drawn only between the treatment groups and controls within each experiment.

Danazol, 20 mg/kg once daily (n = 5). Mean body weights were similar to those of controls and did not

change. Ovaries were similar in gross appearance to those of controls but histologic evaluation was not obtained for this group. Ovarian weights varied widely, ranging between 2.19 and 15.2 gm; however, the mean weights were not statistically different (Fig. 1). Two of five animals developed ascites; the mean score was 1.6 ± 0.4 and was similar to that for controls (Fig. 1). Serial plasma 17β-estradiol levels exhibited marked variability but mean levels were comparable to those of controls; plasma progesterone (Fig. 2) and ovarian homogenate 17β-estradiol and progesterone levels were also similar (Fig. 3). Homogenate prostaglandin F content was significantly lower than that of controls (6.96 ± 2.3 versus 17.59 ± 2.17 pg/mg of protein; $p < 0.01$; Fig. 3).

Danazol, 20 mg/kg twice daily (n = 10). Compared with those of controls, mean body weights and ovarian weights (range 1.74 to 10.1 gm; mean 4.08 ± 0.8 gm) were similar (Fig. 1). No apparent differences in the gross and histologic appearances of the ovaries were observed. Ascites formation was evident in nine animals, and the mean ascites score was comparable to that of controls (Fig. 1). Plasma 17β-estradiol levels were significantly higher on days 5, 7, and 9 while the plasma progesterone response was minimal and did not differ from that of controls (Fig. 2). Ovarian homogenate contents of 17β-estradiol, progesterone, and prostaglandin F were similar (Fig. 3).

Indomethacin, 30 mg/kg once daily (n = 10). These animals had significantly lower body weights on day 9 of the experiment (3.18 ± 0.13 versus 3.56 ± 0.08 kg; $p < 0.01$), while body weights at the start were comparable (Fig. 1). There were no apparent differences in the general health of the animals. External ovarian appearances were similar to those of controls, as were the histologic sections, which showed the presence of hemorrhagic follicles, cystic follicles, and few if any corpora lutea. There was no evidence of ovulation. Mean ovarian weights were similar (Fig. 1). Seven of the 10 animals developed ascites with a mean score of 1.9 ± 0.23 (Fig. 1). Plasma 17β-estradiol levels on days 7 and 9 were significantly depressed, while the plasma progesterone response was minimal (Fig. 2). Ovarian homogenate prostaglandin F was significantly depressed (5.27 ± 1.18 versus 17.59 ± 2.17 pg/mg of protein, $p < 0.01$; Fig. 3).

Comment

In humans, the ovarian hyperstimulation syndrome is a complex series of events comprising marked ovarian enlargement due to multiple follicular and hemorrhagic cysts, multiple corpora lutea, and stromal edema; ovarian vascularity is also increased. In addition enhanced vascular permeability results in acute third-space fluid shifting with its attendant sequelae of hypovolemia, oliguria, renal failure, electrolyte disturbances, hyperviscosity, and hypercoagulability.[1] The third-space fluid shifting and its consequences contribute most significantly to the morbidity and mortality associated with this complication. In these experiments with the rabbit model,[2] there appeared to be wide individual variations in animal responses to a given dosage of Pergonal. Clinically, this is also commonly observed.[1]

We also found that the rabbit can develop an ascites response to gonadotropin hyperstimulation of the ovarian follicles with a minimal to essentially normal luteal response. The observations in the current studies indicate that the ascites response of the hyperstimulated female rabbit shares similarities (ovarian enlargement, multiple hemorrhagic follicles, hyperestrogenemia) and differences (lack of luteal hyperstimulation), as compared with the syndrome in humans.

Hyperstimulation of the ovaries was observed in all three experimental groups as reflected by ovarian enlargement and plasma 17β-estradiol values. In these experiments, mean ovarian weights were approximately tenfold to thirtyfold heavier than those in placebo-treated control animals of comparable body weight. In comparison, rabbits (body weight 2.1 ± 0.1 kg) were given intramuscular injections of saline solution in lieu of gonadotropins as described in the present experiments (placebo controls), the mean ovarian weight was 0.2 ± 0.03 gm (unpublished data).

Mean plasma 17β-estradiol levels observed in the three experiments reported here exceeded values observed in rabbit pseudopregnancy, albeit levels were generally lower in experiment 2, where an FSH-dominant gonadotropin preparation had been used (Fig. 2). No correlation between plasma 17β-estradiol and ascites formation was observed. When we induced pseudopregnancy in 10 rabbits with 100 IU of human chorionic gonadotropin intravenously, peripheral plasma 17β-estradiol levels on days 5, 7, and 9 of pseudopregnancy did not exceed 13 pg/ml and the range of progesterone for comparable phases of pseudopregnancy was 13.31 ± 2.35 to 25.5 ± 4.4 ng/ml (unpublished data). Although plasma progesterone levels in experiment 1 fell within the pseudopregnancy range (Fig. 2), levels in experiments 2 and 3 were much lower, compatible with minimal corpus luteal function. Nevertheless, ascites formation occurred in all three experimental groups. The reason for the higher plasma progesterone response in control animals of experiment 1, compared with those in experiment 3, where both groups received the same amount of exogenous gonadotropin stimulation, is unclear. It may be that experiment 1 controls, having significantly lower mean

body weights (2.67 ± 0.06 versus 3.7 ± 0.10 kg; $p < 0.05$), received relatively larger amounts of Pergonal per kilogram of body weight.

Within each of the experiments, ascites formation had no consistent relationship to ovarian weight. The degree of ascites formation was similar in the less hyperstimulated animals of experiment 2 as compared with that observed in the other two experimental groups. In a review of patients with ovarian hyperstimulation, all of whom had severe ovarian enlargement (ovaries >12 cm), only 36% developed clinically apparent ascites.[1] In yet another report, third-space fluid sequestration in the form of hydrothorax occurred without any significant ovarian enlargement.[2]

Pharmacologic doses of both native GnRH and its agonist analogues have been shown to inhibit steroidogenesis via a direct gonadal action, mediated by specific high-affinity GnRH receptors in rats and guinea pigs.[3, 4] There are data to suggest that rabbit ovaries also contain GnRH or GnRH-like receptors.[5] The dosage of GnRH per kilogram of body weight used in experiment 1 was thirteenfold greater than that used to induce luteolysis of existing corpora lutea in pregnant rabbits and comparable to that previously shown to inhibit ovulation and steroidogenesis in rats.[4, 6] From our data, these very high doses of GnRH did not suppress steroidogenesis in the hyperstimulated rabbit, nor did they influence the other features associated with the ovarian hyperstimulation syndrome. Since natural GnRH has a relatively short half-life, the use of more potent and longer-acting GnRH agonist analogues offers an alternate approach that may yield different results.

Danazol is known to inhibit steroidogenic enzymes in the ovary and adrenal. It blocks aromatization of androgen to estrogen and interferes with progesterone production and luteal function.[7, 8] The doses of danazol we used were at least fourfold the dose that suppressed gonadal steroid production in rats.[7] However, in experiments 2 and 3, danazol, 20 mg/kg once daily or 20 mg/kg twice daily, did not depress steroidogenesis. Indeed, at the higher dose schedule in experiment 3, plasma 17β-estradiol was significantly elevated on days 5, 7, and 9. In patients receiving large doses of danazol, serum 17β-estradiol levels were also elevated.[14] Since danazol competes with 17β-estradiol for estradiol receptors,[13] it is conceivable that increased circulating 17β-estradiol may result from decreased tissue binding. In experiment 3, animals receiving 20 mg/kg of danazol once daily had significantly lower ovarian prostaglandin F as compared with that of controls. However, ovarian prostaglandin F was similar to that of controls in animals receiving a higher dose of danazol (20 mg/kg twice daily). The less hyperstimulated animals in experiment 2 that were given 20 mg/kg/day also did not show significant depression of ovarian prostaglandin F; the reason for the depression of ovarian prostaglandin F observed in experiment 3 is unclear. Despite these endocrine changes seen with the use of danazol, the clinical manifestations of ovarian hyperstimulation syndrome (ascites formation and ovarian enlargement) were unaffected.

It has been suggested that prostaglandins are important mediators of the altered vascular permeability associated with the ovarian hyperstimulation syndrome since inhibition of prostaglandin synthetase by indomethacin suppressed ascites formation in the hyperstimulated rabbit.[1, 10] Our results do not support this contention. With the use of an identical dosage schedule for administering indomethacin (experiments 2 and 3), we were able to demonstrate significant suppression of ovarian prostaglandin F (presumably with other prostaglandins being inhibited as well). Despite this, ascites formation was uninfluenced, suggesting that prostaglandins are not obligatory mediators of the third-space fluid shifting. Indomethacin had no effect on ovarian weight in either experiment. Although steroidogenesis was not significantly affected in experiment 2, indomethacin in the more stimulated animals of experiment 3 significantly lowered plasma 17β-estradiol levels on days 7 and 9. Indomethacin significantly suppressed ovarian progesterone but not 17β-estradiol content. High concentrations of indomethacin can exert pharmacologic effects that are independent of prostaglandin inhibition. These effects include inhibition of cyclic adenosine monophosphate–dependent protein kinase, and indomethacin could interfere with steroid hormone production via this mechanism.[15] Nevertheless, despite its effects on ovarian steroidogenesis and good suppression of ovarian prostaglandin, indomethacin in pharmacologic doses did not influence the clinical features of the ovarian hyperstimulation syndrome (increase in ovarian weight and ascites formation).

In summary, GnRH, danazol, and indomethacin as used in these experiments were not effective in suppressing ovarian hyperstimulation and/or ascites formation. The gonadotropin-induced ascites response in the rabbit occurred despite quite marked variation in the clinical and hormonal response of the ovaries. In contrast to the syndrome in humans, where the ascites response is seen in the presence of follicular and luteal hyperstimulation, the development of ascites in the hyperstimulated rabbit was observed in the absence of significant luteal hyperstimulation.

We are grateful to the Serono Company, Randolph, Massachusetts, for the generous gift of the FSH-dominant gonadotropin preparation and part of the Per-

gonal used in this study. We also wish to thank Mr. Antoni Duleba for his interest and aid in this project.

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The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat

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The purpose of this study was to analyze the changes in maternal uterine blood flow, maternal arterial blood pressure, and maternal heart rate with the intravenous administration of nifedipine in pregnant, unanesthetized pygmy goats. Bolus injections of 2.5 to 45 μg/kg of maternal weight were used. Uterine blood flow did not fall significantly. A transient fall in maternal arterial blood pressure occurred in the first minute after the injection, but the pressure returned to normal after 5 minutes. Maternal heart rate increased immediately after the bolus of nifedipine and returned toward the control value by 30 minutes. (AM J OBSTET GYNECOL 1986;154:1160-3.)

Key words: Uterine blood flow, pregnant pygmy goat, calcium channel blocker

Nifedipine, a calcium channel blocker, prevents the entry of ionized calcium in the slow channels of the cell membrane and results in an inhibition of smooth muscle contraction.[1,2] Its use in cardiology has been mostly in the treatment of angina pectoris, causing vasodilation of the coronary vascular bed, and in hypertension, causing an inhibition of vascular smooth muscle tone.[3] Since there is a need for a potent tocolytic agent with minimal side effects, some investigators have examined the effects of this drug on uterine contractions. In vitro studies done on uterine strips showed that nifedipine inhibited oxytocin-induced contractions.[4,5] Administration of nifedipine to women with dysmenorrhea,[6] to women undergoing midtrimester abortion induced by prostaglandin $F_{2\alpha}$,[7] and to women with postpartum uterine contractions[8] resulted in successful inhibition of uterine activity. Because of these observations nifedipine was used successfully in the treatment of 10 patients in premature labor.[9] However, since both uterine and vascular smooth muscle are affected by nifedipine, hemodynamic side effects are of concern. When a bolus

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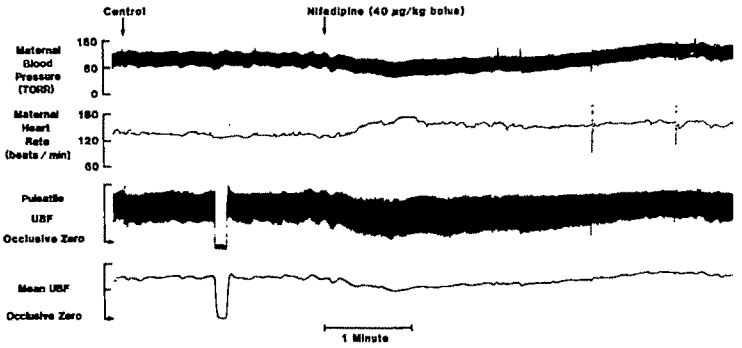


Fig. 1. Strip chart recording of maternal arterial pressure, maternal heart rate, and uterine blood flow *(UBF)*. A control period is indicated, during which an occlusive zero was obtained. An intravenous bolus of 40 µg/kg was given during 1 minute, as indicated. A fall in maternal arterial pressure and in uterine blood flow was observed. Maternal heart rate increased immediately after the bolus injection.

of nifedipine was given to nonpregnant unanesthetized dogs, a 30% fall in mean arterial pressure was seen.[10] Infusion of nifedipine at 10 µg/kg/min in pregnant ewes resulted in a significant fall in maternal blood pressure and systemic vascular resistance (see reference 14). Such a fall in pressure in a pregnant animal might significantly affect maternal uterine blood flow,[11] reduce placental perfusion, and thus result in a deleterious effect on the fetus. The purpose of this study was to analyze the changes in uterine blood flow and in maternal blood pressure and heart rate during intravenous bolus administration of nifedipine in the pregnant pygmy goat.

Material and methods

Six time-bred pregnant pygmy goats weighing between 15 and 40 kg, with a mean gestational age of 126 ± 2 days (term = 150 days), were used in this study. Each was fasted overnight and given atropine. Anesthesia was induced with intravenous barbiturate. After intubation, the goats were ventilated with halothane (1% to 2%) and a balance of 50% oxygen and 50% nitrous oxide. A low midline incision was made and the pregnant horn was located. The uterine artery supplying that horn was cleared of connective tissue to allow placement of an electromagnetic flow probe transducer with a lumen diameter between 4 and 6 mm (Carolina Medical Electronics). An inflatable occluder, made of silicone rubber, was placed 1.0 to 1.5 cm distal to the flow probe. The carotid artery and the jugular vein of the goat were isolated and cannulated nonoccclusively with polyvinyl catheters. All catheters and wires were routed subcutaneously to the maternal flank and kept in a cloth pouch sewn to the skin.

At least 3 days passed before the animals were studied. Nifedipine (supplied by Dr. N. Gerber, Pfizer, Ltd.), in a powder form, was prepared on the morning of the experiment in a light-protected environment. Nifedipine, 5 or 10 mg, was dissolved in 20 ml of 95% ethyl alcohol and 30 ml of distilled water, yielding a 100 µg/ml or a 200 µg/ml solution, respectively. The flow probe wires were connected to the flowmeter (Model 501, Carolina Medical Electronics), which was then balanced and nulled. Both pulsatile and mean (0.3 Hz low-pass filter) uterine blood flow were recorded on a strip-chart recorder (Model R611, Beckman Instruments). The maternal arterial catheter was connected to a pressure transducer (Statham Model 23Db), which was positioned at the midthoracic level of the goat. Maternal heart rate was determined by a cardiotachometer (Beckman Model 9857) triggered by either the arterial pressure pulse or the flow.

When the instrumentation was operational, the occluder was inflated for a 10-second period. This occlusive zero was used for the no-flow reading and was obtained after each study. Only trials in which the occlusively determined zero flow outputs did not differ by more than 5% with reference to the output determined during an initial 30- to 40-minute control period were accepted for analysis. After the control period, a bolus of nifedipine was infused into the maternal jugular vein. The initial dose was either 2.5 or 5 µg/kg and this was followed by increased doses of the drug. We allowed 40 to 60 minutes to elapse between each bolus of nifedipine and performed three to five studies per day in each animal. In all, 102 studies were performed in the six animals.

Uterine blood flow, mean arterial blood pressure (systolic pressure + 2 diastolic pressure/3)[12] and maternal heart rates were transcribed from the chart records at 10-second intervals for the first 5 minutes after the injection of nifedipine. These were averaged for the first minute (immediate response) and separately for the second to the fifth minute (early response). The data were transcribed at 30-second intervals from the fifth to thirtieth minute and averaged (late response).

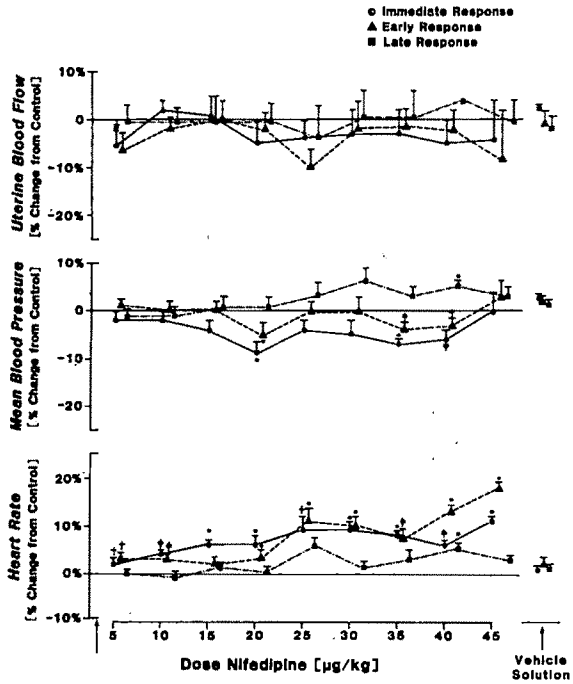


Fig. 2. Uterine blood flow, maternal arterial pressure, and maternal heart rate as a function of the bolus dose of nifedipine at three periods after the drug: immediate (0 to 1 minute), early (1 to 5 minutes), and late (5 to 30 minutes). Each point represents the average of seven to nine studies. (* = $p < 0.01$; † = $p < 0.05$; ‡ = $p < 0.02$.)

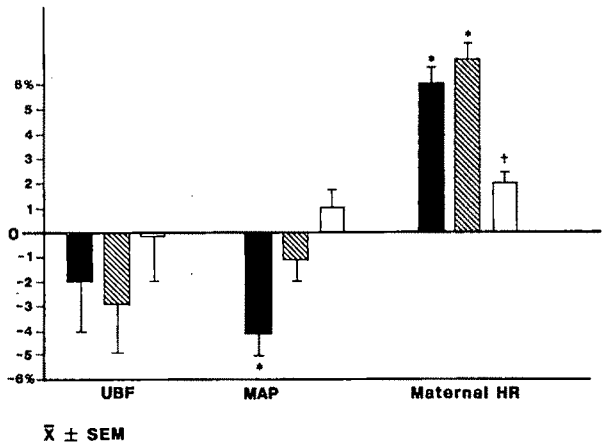


Fig. 3. Data obtained by pooling all doses of nifedipine in Fig. 2 showing percent of change from control for uterine blood flow *(UBF)*, maternal arterial pressure *(MAP)*, and maternal heart rate *(HR)*. *Solid bars* = Immediate response (0 to 1 minute); *hatched bars* = early response (1 to 5 minutes); *open bars* = late response (5 to 30 minutes). (* = $p < 0.01$; † = $p < 0.05$.)

Uterine blood flow is expressed as a percentage of the control blood flow and not in terms of absolute blood flow, as only a portion of the total uterine blood flow was measured and because of the inherent difficulty in calibrating chronic flow probes.[13] Mean arterial pressure and heart rate are also expressed as a percentage of the control value obtained for each study day. Data for each dose of nifedipine were averaged and compared with control values by a paired *t* test with a two-tailed table of significance.

Results

During the control period mean arterial pressure in these goats averaged 85 ± 1 torr (±SEM) and heart rate was 131 ± 1 bpm. By definition, the control uterine blood flow was 100%. Fig. 1 shows the response of maternal blood pressure, heart rate, and uterine blood flow to a bolus of 40 µg/kg of nifedipine. The fall in blood pressure and uterine blood flow accompanied by a rise in heart rate observed in the immediate and early periods after the bolus was not typically as pronounced as seen in this study even at the higher doses of nifedipine.

In Fig. 2, the average changes in uterine blood flow and in maternal arterial pressure and heart rate observed for boluses of nifedipine over a range of 5 to 45 µg/kg are depicted. There was no significant change in uterine blood flow at either the immediate (1 minute), the early (2 to 5 minutes), or the late (5 to 30 minutes) period after the drug. Maternal arterial pressure fell by 6% to 10% at nifedipine doses of 20, 35, and 40 µg/kg. This significant decline was seen during the immediate and early time periods and did not persist beyond 5 minutes. Maternal heart rate increased significantly during the 5 minutes after the bolus and the increase was more pronounced at a dose of ≥25 µg/kg. Heart rate returned toward control values in the next 25 minutes. Fig. 2 shows the infusion of the vehicle used in these studies had no effect on uterine blood flow, arterial pressure, or heart rate.

In Fig. 3, we have grouped the results at all doses of nifedipine. The fall in mean arterial pressure was confined to the first minute after the nifedipine bolus and the maternal tachycardia was seen to persist for 30 minutes although the change seen in the late period was minor.

Comment

A bolus infusion of nifedipine (20 µg/kg) in unanesthetized dogs produced a 30% fall in mean arterial pressure.[10] If a similar fall in blood pressure were to occur in a pregnant animal and the uterine circulation did not dilate, a corresponding fall in uterine blood flow would be anticipated.[11] With the use of bolus infusions up to twice that which caused hypotension in the dog, we have observed only a minor fall in blood pressure in pregnant pygmy goats. In this respect, our results are consistent with those recently reported by Golichowski et al.[14] in pregnant sheep. The study de-

sign differed from ours in that Golichowski et al. used 30-minute constant infusions of nifedipine over the range 1 to 10 μg/kg/min. They observed a 12% fall in blood pressure at the highest dose.

Although a transient fall in uterine blood flow was seen in some studies (Fig. 2), when flow was averaged, there was no significant fall to this organ. This indicates that the tissues supplied by the vasculature distal to our flow probe dilated at least to the extent that perfusion pressure fell. Blood flow measured by a probe on the uterine artery goes to two vascular beds, the placenta and the myoendometrium, which may show different responses. In the past we have shown that the fall in uterine blood flow that occurs during exercise in the pregnant pygmy goat[12] is seen primarily in the myoendometrial bed. In these studies we used microspheres to separate blood flow in the uterus into its two components and found that placental or cotyledonary flow fell <10% while that to the myoendometrium declined to less than half of the resting level. It should be noted that the response in both these vascular beds was in the same direction. It is possible, in the present experiments with nifedipine, that a fall in placental blood flow was balanced by an increase in flow to the myoendometrium, such that no change was seen in total uterine blood flow. However, on the basis of the previous studies cited above, in which both placental and nonplacental vasculatures showed a qualitatively similar response to a given perturbation, we feel that it is unlikely that the uterine circulation underwent a combined constriction and dilation in its two vasculatures after the bolus injection of nifedipine.

In conclusion we have shown that the uterine circulation of the pregnant pygmy goat does not experience any change in flow rate after bolus injections of the calcium channel blocker nifedipine up to 45 μg/kg.

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CORRESPONDENCE

Goiter in pregnancy

To the Editors:

The article by Long et al. (Long TJ, Felice ME, Hollingsworth DR. Goiter in pregnant teenagers. AM J OBSTET GYNECOL 1985;152:670) addresses the question of a possible goitrogenic effect of pregnancy. Clearly, many of the discrepancies between studies can be explained by geographic, racial, and environmental differences in the study populations. Thus urinary iodine excretion differs widely from country to country and also within countries.

We, however, wish to draw attention to the inexactness and irreproducibility of the clinical evaluation (palpation) of thyroid size[1] and stress that such an evaluation does not with any reliability allow a quantitative evaluation of thyroid size. Such an evaluation could have been achieved by use of ultrasound[2] and is necessary if one wishes to demonstrate alterations in thyroid size in a range of thyroid volume where no goiter is evident. Therefore, an increase in thyroid volume could well have been demonstrated in spite of the failure to demonstrate a difference in goiter frequency between pregnant and nonpregnant subjects.

Recently a number of factors have been found to influence thyroid size. Thus sex, age, and body weight[2] as well as the presence of liver[3] and kidney disease[4] and possibly excessive alcohol intake[3] influence thyroid volume determination. Cigarette smoking is associated with a marked increase in goiter frequency[5] and the menstrual cycle in healthy women is associated with cyclic alterations of thyroid volume of more than 50% (unpublished data). If a valid comparison is to be made between pregnant and nonpregnant subjects it is mandatory that the two groups are comparable with respect to these variables.

From the data presented by Long et al., in our opinion the question whether thyroid size is increased during pregnancy has not been answered. A longitudinal study of ultrasonically measured thyroid volume in pregnant women might possibly give this answer.

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Reply

To the Editors:

We would like to express our appreciation to Drs. Hegedüs, Rasmussen, and Karstrup for their interest in our study on the occurrence of goiter in pregnant and nonpregnant female adolescents. We feel they have understood the purpose and scope of our investigation.

We feel that the likelihood of iodine deficiency in our region is unlikely and believe that it would not have been experimentally useful or clinically practical to measure urinary iodine excretion in our patient population.

It is our opinion that although ultrasound assessment of the thyroid glands (to confirm enlargement) in our goitrous adolescent population would have been scientifically elegant, it would not have been cost-effective for our patient population and thus was not clinically justified.

Instead, we elected to be less exact and to adopt the current internationally accepted World Health Organization standards for the determination of thyroid size by palpation.

We feel that our observations have scientific merit and are of clinical usefulness for practicing physicians.

Thank you for your interest! We look forward to further entries in the medical literature regarding the validity of goiter diagnosis by palpation, and it would certainly be of interest to thyroidologists and obstetricians in our country if such an extensive ultrasound study could be performed on pregnant teens in Denmark.

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Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein

To the Editors:

Recently, Sawada et al.[1] reported on determination of serum immunosuppressive acidic protein in patients with ovarian cancer. The authors concluded that measurements of this protein are a valuable adjunct to the conventional diagnostic methods and a useful procedure for the monitoring of the results of treatment.

Furthermore, the authors pointed out that immunosuppressive acidic protein differs from α_1-acid glycoprotein. This may apply to physicochemical characteristics such as isoelectric point, molecular weight, and carbohydrate content,[2] but according to Tamura et al.[2] the behavior of immunosuppressive acidic protein in immunodiffusion and immunoelectrophoresis is identical to that of normal human α_1-acid glycoprotein.

Sawada et al. used radial immunodiffusion for the measurement of immunosuppressive acidic protein without discussing whether they quantitated immunosuppressive acidic protein, α_1-acid glycoprotein, or a mixture of the two glycoproteins. In order to elucidate this problem we have investigated 33 serum samples from 15 patients who had or were thought to have cancer of the ovary.

Each of the samples was tested by radial immunodiffusion in two agar plates; one contained anti–immunosuppressive acidic protein (commercially available from Sanko Junyaku Co. Ltd., T.M.M. Bldg., 1-10-6 Iwamoto-Cho, Chiyoda-Ku, Tokyo 101, Japan) and the other anti–α_1-acid glycoprotein (commercially available from Hoechst, Verkauf Pharma, Diagnostika, D-6230 Frankfurt [Main] 80, West Germany). All the samples formed ring precipitates in both plates. The diameter of the precipitates was measured and calculated as micrograms per milliliter by means of standard curves. The content of immunosuppressive acidic protein and α_1-acid glycoprotein in each sample was marked by one single point in a coordinate system having the immunosuppressive acidic protein and α_1-acid glycoprotein values as ordinate and abscissa axis, respectively. The results of the two methods are shown in Fig. 1. The values appear highly correlated ($r = 0.994$). A linear regression analysis by means of the method of least squares gave the following equation: $y = 0.612x - 28.795$. The standard deviation of the slope was 0.010. Measurements of standards with known content of marker by both methods gave values in agreement with the equation.

In conclusion, single radial immunodiffusion performed in the commercially available plates used by us did not allow us to distinguish between immunosuppressive acidic protein and α_1-acid glycoprotein. This finding is in agreement with that of Tamura et al.,[2] who concluded that the behavior of immunosuppressive acidic protein in immunodiffusion is indistinguishable from that of normal human α_1-acid glycoprotein.

Therefore, which of the two plates should be preferred for marker determination in ovarian cancer depends on criteria other than the ability to distinguish between immunosuppressive acidic protein and α_1-acid glycoprotein. However, the importance of immunosuppressive acidic protein in the clinical assessment of

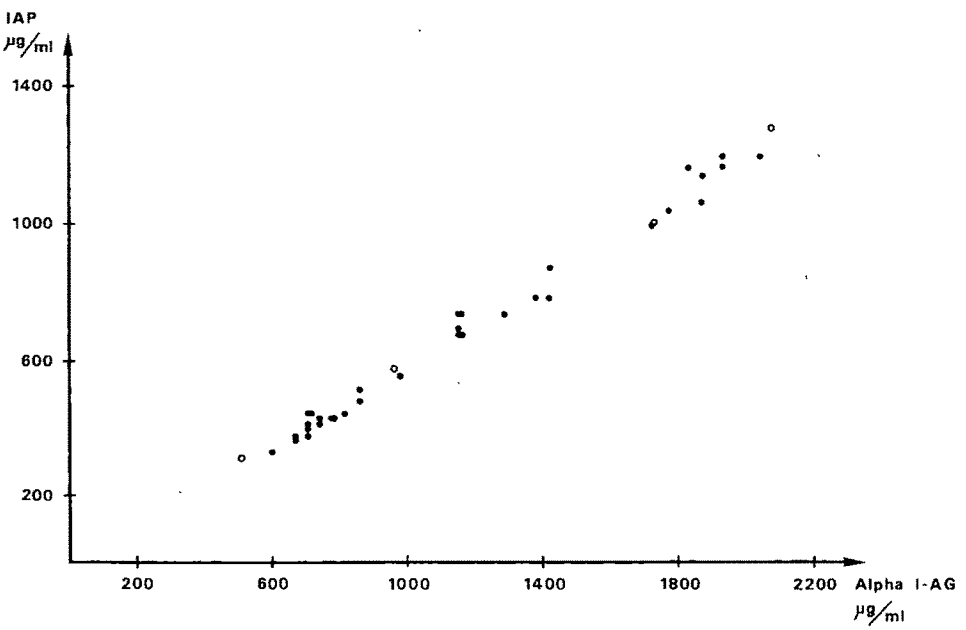


Fig. 1. Corresponding values of immunosuppressive acidic protein and α_1-acid glycoprotein measurements performed on the same serum sample. *Open circles* = four standards; each point represents the mean value of five replicates on each type of plate. *Solid circles* = 33 serum samples. *IAP* = Immunosuppressive acidic protein; *Alpha 1-AG* = α_1-acid glycoprotein.

patients with ovarian cancer seems to be a problem that needs further investigation.

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Measurements of delta optical density at 450 nm in SS disease

To the Editors:

I read with interest the recent article by Lindsay and Lupo concerning false elevations of amniotic fluid delta optical density at 450 nm in patients with SS disease and hyperbilirubinemia (AM J OBSTET GYNECOL 1985; 153:75). I first reported this observation in a sickle cell patient in 1978 (AM J OBSTET GYNECOL 1978;130:103). In addition, two additional cases have recently been reported from our laboratory.[1]

I think these collective cases should serve as adequate warning to obstetricians who are managing patients with potential maternal-fetal blood group incompatibilities and who coincidentally have maternal hyperbilirubinemia. The need for intrauterine transfusion and/or early delivery should be very cautiously deliberated.

I would like to make one last comment about the paper of Lindsay and Lupo. In their last paragraph they state that this false elevation of bilirubin level in the amniotic fluid documents indirectly the passage of bilirubin from the maternal to the fetal compartment. I do not think this is necessarily the case. The normal transport of bilirubin is from the fetal to the maternal compartment. An elevation in the fetal compartment as a result of a maternal elevation probably reflects a buildup in the fetal compartment because of inhibition of flow from the fetal to the maternal compartment rather than a flow from mother to fetus. Except in extreme cases of acute maternal hyperbilirubinemia, I doubt that the net flow is ever from the maternal to the fetal compartment.

George H. Nelson, Ph.D., M.D.
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REFERENCE

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Reply

To the Editors:

I would like to thank Dr. Nelson for his interest in our report. He presents a plausible mechanism for the elevation of bilirubin in the amniotic fluid, which unfortunately cannot be substantiated by experimental data. Lipsitz et al.[1] presents data which may indirectly support the passage of bilirubin from the maternal to fetal compartment.

In their report they noted that the level of cord blood and maternal serum unconjugated bilirubin was similar. If amniotic fluid elevation was from inhibition of transport, one would expect the level of unconjugated bilirubin in the cord blood to be higher than that in the maternal serum. In the Lipsitz report this was not the case, as the cord blood bilirubin was 9.8 mg/ml and that in maternal serum at the time of delivery was 11 mg/ml. Unfortunately in our report we did not have access to cord blood bilirubin levels. Certainly, further study is warranted.

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REFERENCE

1. Lipsitz PJ, Flaxman LM, Tartow LR, et al. Maternal hyperbilirubinemia and the newborn. Am J Dis Child 1973;126:525.

Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura

To the Editors:

We would like to comment on the statement of Martin et al. (Martin JN, Morrison JC, Files YC. Autoimmune thrombocytopenic purpura: Current concepts and recommended practices. AM J OSTET GYNECOL

1984;150:86.) discouraging breast-feeding in thrombocytopenic infants born to women with autoimmune thrombocytopenic purpura.

A recent patient of ours with autoimmune thrombocytopenic purpura and previous splenectomy was referred to our center for management of the disease during her third pregnancy. She had a first child by vaginal delivery with neonatal thrombocytopenia and cerebral hemorrhage with persistent neurologic deficit, and a second child died after vaginal delivery with intracranial hemorrhage. She received steroids in the last 3 months of her pregnancy because of severe thrombocytopenia (<10,000/mm^3) and started labor at 34 weeks. The premature baby was delivered by cesarean section and showed disseminated bruises. The baby's platelet count was <10,000/mm.^{3} Platelet transfusion was given and exsanguination transfusion was performed, corticosteroid therapy was started, 1 mg/kg/day, and platelet transfusions were continued for 2 weeks. The clinical status deteriorated because of sepsis and respiratory distress. Antibiotics and oxygen were administered, and the clinical situation improved. The baby was first fed by nasogastric tube with the mother's milk when he was 5 days old; when he was able to suck, breast-feeding was started without deterioration of his platelet count. Corticosteroids were stopped after 2 weeks and breast-feeding was continued; the platelet count remained at 145,000/mm^3 and no more platelet transfusions were required.

Although it is only one case, we have evidence that in this severely thrombocytopenic baby, breast-feeding did not accentuate the thrombocytopenia. In a letter of Kelemen et al.[1] there is only a suggestion that platelet counts in the babies reported by Jones et al.[2] were lowest between the second day and fourth day as a result of breast-feeding, but there is no proof that this was the cause. In fact, no absorption of breast milk antibodies could be demonstrated in humans,[3] so we think that more cases are needed to establish whether breast-feeding is safe or not in these cases.

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3. Hanson LA. The mammary glands as an immunological organ. Immunol Today 1982;3:168.

Reply

To the Editors

We would like to thank Drs. Meschengieser and Lazzari for communicating their recent experience with a parturient who had autoimmune thrombocytopenic purpura after splenectomy and who, after cesarean birth of her thrombocytopenic preterm infant, contributed breast milk and later breast-fed without apparent adverse effect on the neonatal platelet count.

Their case report serves to reinforce the likelihood of severe fetal thrombocytopenia in the splenectomized parturient with autoimmune thrombocytopenic purpura. Their finding that breast-feeding beginning on the fifth day did not worsen the neonatal thrombocytopenia is valuable information. We agree that further study and discussion is needed to clarify this issue and suspect that the benefits of breast-feeding in the preterm infant especially may outweigh theoretical or actual risk.

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Mobiluncus-specific antibodies in a postoperative infection

To the Editors:

Mobiluncus, a bacterium closely related to bacterial vaginosis, has during the last year been reported by two different authors to give deep infections.[1, 2] Serologic studies have, however, not yet been able to demonstrate antibodies against *Mobiluncus* in patients with bacterial vaginosis.[3] In this letter we present the result of the case of an abdominally hysterectomized woman with postoperative infection of *Mobiluncus,* in which we have used an indirect immunofluorescence assay to measure *Mobiluncus*-specific antibodies in serum.

Whole untreated bacterial cells were used as antigen, and screening for the isolate with highest antigenic activity was performed by enzyme-linked immunosorbent assay. The chosen isolate, *KB,* gave a reaction two twofold dilutions higher than the type strain of *Mobiluncus curtisii* and five twofold dilutions higher than the isolate with the poorest reaction. In indirect immunofluorescence assay approximately 10^5 bacteria per milliliter from 3-day-old cultures suspended in 0.01mol/L of phosphate-buffered saline solution were placed on microscopic slides, dried in air, and used without fixation. To each well on the microscopic slide 20 μl of serum diluted 10-fold and further diluted twofold (10, 20, 40, etc.) was applied. After incubation in a moist chamber for 20 minutes at 37° C, the slides were washed in phosphate-buffered saline solution and the slides were stained with fluorescein isothiocyanate–labeled sheep antihuman immunoglobulin (National Bacteriological

Laboratory, Stockholm, Sweden). As controls slides with only conjugates and with serum diluted in a 1 : 10 ratio and the conjugate eliminated were used. After incubation for 20 minutes in a moist chamber at 37° C the slides were washed in phosphate-buffered saline solution 10 minutes and observed for fluorescence in a Zeiss fluorescence microscope with use of Ploem optics and filters. The reactions were conventionally graded: −, 1+, 2+, 3+, and 4+. Reactions graded 2+ to 4+ were recorded as positive.

The patient, a 43-year-old woman, para 2, in 1980 had a cervical dysplasia and in 1981 underwent a minilaparotomy for the purpose of sterilization. In March 1985 an abdominal hysterectomy was performed because of menorrhagia. The histopathologic examination showed leiomyoma of the uterus and an unspecific cervicitis. On the fourth postoperative day the patient became febrile and had low pelvic pain. Examination revealed a foul-smelling, bloodstained discharge and a small palpable induration in the top of vagina. *Mobiluncus* species and gram-positive anaerobic cocci were isolated from the top of vagina. The patient was treated with metronidazol, 500 mg three times daily, and amoxicillin, 700 mg three times daily, for 7 days. She became afebrile and clinically well and was discharged from the hospital. Four weeks later she returned to the hospital with subfebrility and foul-smelling discharge for the previous week. Presence of *Mobiluncus* was demonstrated by a wet-smear study, and gynecologic examination was normal except for a small mass in the top of vagina, which was demonstrated by ultrasound as a cystic cavity, 2 cm in diameter. She received additional treatment with metronidazol, 500 mg three times daily, but this time for 10 days, since we have seen therapy failure with the shorter duration of treatment.[4] The patient recovered from the symptoms and wet-smear and gynecologic examinations after 1 and 5 months revealed no signs of *Mobiluncus* infection. Blood samples were collected for analysis of antibodies against *Mobiluncus*.

The first serum, from 56 days after the operation, gave a titer of 1:320 when tested for IgG antibodies; the second taken 64 days later gave a titer of 1:640. Serum taken 62 additional days later showed reduction in titer to 1:320, and the last serum after an additional 30 days gave a titer of 1:80. The reduction, in titers here is significant, since they are much higher than controls or what other authors found in serologic studies of patients with bacterial vaginosis.[5] A remarkable finding is the specificity of the antibodies, showing reactivity with *Mobiluncus curtisii* but not with *Mobiluncus mulieris* and, in addition, a possible means to discriminate between different isolates among *Mobiluncus curtisii*, a sensitivity which is higher than serum from immunized rabbits.[5] The sera did not react with a wide panel of unrelated bacteria.

These findings indicate the need for further clinical and laboratory investigation in order to clarify whether infections of *Mobiluncus* have to be taken into consideration as an etiologic agent of infections of the female genital tract other than bacterial vaginosis.

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Finger extension of transverse uterine incision

To the Editors:

I agree with the article by Dr. Jovanovic (Incisions of the pregnant uterus and delivery of low-birth weight infants. Am J Obstet Gynecol 1985;971:971-4) except his last sentence, "Thus the fingers should never be used to tear the pregnant uterus transversely . . .."

The rationale for the finger technique was (1) that it enabled one to separate the transverse fibers of the lower uterine segment without cutting the fibers and (2) that it is technically easier to separate the fibers with the fingers than by cutting.

When the fibers are cut, bleeding is increased and the procedure toward the operator must be done backwards with the Bendage scissors—or else the assistant must assist in that portion of the operation; if the assistant is not trained, then the operator must do it himself.

The finger technique is more efficient also because the fingers extend the incision to the limits of the presenting part and not beyond. This affords the minimal length of the incision necessary.

The paper is provocative and timely. It also includes the method for delivering the small fetus. Vaginal delivery is still the best method.

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Reply

To the Editors:

I would like to thank Dr. Gerace for his interest and support of my concept as presented in the article.

In the paper I explained why the so-called "finger technique" created the smallest incision on the uterus and pointed to serious consequences of this small opening on the uterus. These serious complications are injuries of parametrial vessels, involuntary extension of the incision, and midline uterine incision. Bleeding from the cut or finger tear of the low uterine segment is irrelevant as a serious complication.

In addition to what I already said in the paper, I would like to add one point. If we have a hollow, elastic body, a shape such as the uterus, and we make a small transverse incision with a knife and try to extend this incision to the side, we will see that this is impossible because of the elasticity. As an end result there will be a small tear, which is the main point of my paper.

Radoslav Jovanovic, M.D.

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Lupus anticoagulant and pregnancy

To the Editors:

A large number of publications have confirmed the association between the lupus anticoagulant and pregnancy complications.[1-5] Moreover, a high dose of prednisone (40 to 60 mg/day) in combination with aspirin (75 mg/day) seems to improve the outcome in pregnant women with a bad obstetric history involving the lupus anticoagulant. However, this high dose of steroids has been associated with a number of complications.

We now have complete follow-up of three women with lupus anticoagulant, in whom all eight previous pregnancies had been unsuccessful. Administration of prednisone in a dose sufficient to suppress the lupus anticoagulant activity (10 to 20 mg/day) combined with aspirin (20 mg/day) resulted in the birth of a normal weight and healthy term newborn infant in all three cases. There were no major complications in the corticosteroid therapy. Three more cases are currently being treated in the same way with a normal evolution of pregnancy as of this date.

There is no doubt that the lupus anticoagulant is an important entity in reproductive failure and that the preliminary results of therapeutic intervention are promising. However, the pathophysiologic background needs further clarification. In addition, controlled multicenter prospective studies on treatment need to be done to prevent growing confusion and controversy.[6] These studies must be well designated with regard to patient collection and treatment protocol. We suggest that groups interested in initiating or contributing to such studies identify themselves and exchange protocols.

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REFERENCES

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A sonographic sign for fetal Down's syndrome

To the Editors:

We have read with interest the report by Benacerraf et al. (A sonographic sign for the detection in the second trimester of the fetus with Down's syndrome. Am J Obstet Gynecol 1985;151:1078-9) identifying increased skin or soft tissue thickening at the neck as a sign indicating a high risk for Down's syndrome in the fetus. In their introductory sentences the authors convey the idea that this sign is the sole sonographic feature accompanying this syndrome, but there are many reports showing the relationship between Down's syndrome and a large number of echographic findings. Tchobroutsky et al.[1] showed trisomy 21 to be correlated with early (before week 20) fetal growth delay, an association which we recently observed as well. We have seen brachycephaly in Down's syndrome, and Collet et al.[2] found an abnormal biparietal/femur length proportion that was caused by femoral shortness occurring before week 20 in trisomy 21. Finally, we have repeatedly pointed to the high rate of association of trisomy 21 with duodenal atresia (100% in our experience), whereas this association is much less frequently

Table I. Echographic features associated with trisomy 21

Maternal age (yr)	*Gravidity/parity*	*Echographic features*
31	4/2	Low profile growth
43	15/12	Double-bubble image, hydramnios, duodenal atresia
18	1/0	Double-bubble image, hydramnios, duodenal atresia, hydrothorax
40	2/1	Double-bubble image, hydramnios, duodenal atresia
33	4/2	Double-bubble image, hydramnios, duodenal atresia, hydrothorax
21	1/0	Early growth delay

found together with the other forms of intestinal atresia.[3, 4]

In our series of more than 80 echographically diagnosed fetal malformations, 16 were intestinal malformations and abdominal wall defects. Of these 16, two were oesophageal atresias, one was jejunoileal atresia, and four were duodenal atresias, all of the last four occurring in trisomy 21 babies. One trisomy 21 baby showed early growth retardation and another low profile growth. Thus, of our trisomy 21 fetuses, six displayed echographic features related to this condition (Table I).

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REFERENCES

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Relative placental size in growth-retarded infants

To the Editors:

In a recent study of infants born to women with preeclampsia, Teasdale suggested that placental growth was not retarded as the placental weight to infant weight ratio was similar to that found in normal control infants (Histomorphometry of the human placenta in maternal preeclampsia. AM J OBSTET GYNECOL 1985; 152:25-31). However, this conclusion may be misleading because of the choice of infant weight as the denominator in assessing relative placental weight. The birth weight of the five study infants was more depressed than their crown-heel length or head circumference, suggesting that they were wasted. This proposed loss of soft tissue is supported by a calculated mean ponderal index of 2.26 in the study infants compared to 2.56 in the control infants. A loss of body weight resulting from wasting would tend to keep the placental weight/infant weight ratio normal, as has been argued previously.[1, 2]

It is advocated that infant length or head circumference, which are more stable measurements of infant size at birth, are preferable denominators when assessing relative placental size. For example, the calculated mean placental weight/infant length ratio for the study and control infants is 9.06 and 9.23, respectively. This difference strongly suggests that infants born to women with preeclampsia may indeed have relatively underweight placentas.

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REFERENCES

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Reply

To the Editors:

I appreciate Dr. Woods' interest in my paper, and I understand his comments concerning the use of other denominators than fetal weight to assess relative placental size. However, in the case of this study Dr. Wood has misinterpreted the data, and therefore his comments are not relevant to the conclusions raised by this investigation. For example, the ratios of placental and parenchymal weights, total trophoblast nuclei content, total villous and capillary surface areas, and intervillous space volume to fetal weight were all significantly "higher" in the study group compared to the control group (Table III). In contrast, the placental weight/infant length ratios for the study and control infants were 9.07 ± 0.72 (mean $\pm$ SD) and 9.23 ± 0.97 ($p > 0.05$), respectively. Therefore, in contrast to Dr. Woods' comment, these results suggest that placental growth was not retarded either in absolute or in relative values in the study group of preeclamptic mothers reported in this paper.

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Books received

Biology of Ovarian Follicles in Mammals. Sardul S. Guraya. 320 pages, illustrated. New York, 1985, Springer-Verlag New York, Inc. $69.50.

Clinical Aspects of Perinatal Medicine. Volume 1. Edited by Manohar Rathi. 387 pages, illustrated. New York, 1985, Macmillan Publishing Company. $49.95.

Clinics in Obstetrics and Gynaecology. Volume 12/Number 2. Guest Editor Shlomo Raz. 244 pages, illustrated. Philadelphia, 1985, W. B. Saunders Company. $24.00.

Confronting the Malpractice Crisis. Guidelines for the Obstetrician-Gynecologist. Edited by Daniel K. Roberts, Jeffrey A. Shane, and Margaret L. Roberts. 304 pages. Kansas City, Kansas, 1985, Eagle Press, Inc. $50.00.

Drug Therapy During Pregnancy. Edited By Tom K. A. B. Eskes and Mieczyslaw Finster. 229 pages. Stoneham, Massachusetts, 1985, Butterworth Publishers. $79.95.

Fetal Alcohol Exposure and Effects. A Comprehensive Bibliography. Compiled by Ernest L. Abel. 309 pages. Westport, Connecticut, 1985, Greenwood Press. $45.00.

Fetal Heart Rate Monitoring. Clinical Practice and Pathophysiology. Edited by W. Kunzel. 244 pages, illustrated. New York, 1985, Springer-Verlag New York, Inc. $44.00.

Health Needs of Women as They Age. Sharon Golub and Rita Jackaway Freedman. 122 pages. New York, 1985, The Haworth Press, Inc. $24.95.

Intrauterine Contraception. Advances and Future Prospects. Gerald I. Zatuchni, Alfredo Goldsmith, and John J. Sciarra. 504 pages, illustrated. Philadelphia, 1985, J. B. Lippincott Company. $25.00.

Issues and Reviews in Teratology. Volume 3. Edited by Harold Kalter. 299 pages. New York, 1985, Plenum Publishing Corporation. $49.50.

Lymphatic Imaging. Lymphography, Computed Tomography and Scintigraphy. Second Edition. Melvin E. Clouse and Sidney Wallace. 526 pages, illustrated. Baltimore, 1985, Williams & Wilkins.

The Pharmacological Basis of Therapeutics. Seventh Edition. Alfred Goodman Gilman, Louis S. Goodman, Theodore W. Rall, and Ferid Murad. 1839 pages. New York, 1985, Macmillan Publishing Company. $65.00.

Radiation Oncology. Stewart M. Jackson. 264 pages, illustrated. St. Louis, Missouri, 1985, Warren H. Green, Inc. No price listed.

Risks of Labour. Volume 2. Guest Editor J. W. Crawford. 214 pages, illustrated. New York, 1985, John Wiley & Sons. No price listed.

Tumors and Tumorlike Conditions of the Ovary. Edited by Lawrence M. Roth and Bernard Czernobilsky. 296 pages, illustrated. New York, 1985, Churchill Livingstone, Inc. $59.00.

Ultrasound Annual—1985. Edited by Roger C. Sanders and Michael C. Hill. 381 pages, illustrated. New York, 1985, Raven Press.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Three Postgraduate Gynecology Courses: Advanced Gynecologic Oncology, September 5-7, 1986; Advances in Gynecology, September 8-10, 1986; Reproductive Endocrinology, September 11-14, 1986; Massachusetts General Hospital, Boston, Massachusetts. Contact: Harvard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115.

First Annual Postgraduate Course in Obstetrics and Gynecology, May 30-31, 1986, The Allentown Hilton and Conference Center, Allentown, Pennsylvania. For further information, contact: Sze-ya Yeh, M.D., Department of Obstetrics and Gynecology, The Allentown Hospital, 17th and Chew Streets, Allentown, PA 18102. Tel.: (215) 778-2412.

Gynecologic Surgical Techniques, June 12-14, 1986, Chicago, Illinois. For additional information, contact the Registrar's office. Toll-free number: Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

The 11th Annual Perinatal Seminar, July 17 and 18, 1986, Windmill's Ashland Hills Inn, Ashland, Oregon. For further information contact the Education Department, Rogue Valley Medical Center, 2825 Barnett Road, Medford, OR 97504. Tel.: (503) 770-4148.

1986 Canada West Medical Congress, June 29–July 5, 1986, Hyatt Regency Hotel, Vancouver, British Columbia, Canada. For additional information call: (604) 736-5551, Conventions Department, British Columbia Medical Association.

Prenatal Diagnosis: Principles and Clinical Applications, July 26-31, 1986, Snowmass, Colorado. Sponsored by Department of Obstetrics and Gynecology, Texas A & M College of Medicine. For further information contact: Sharon Stermer, CME Office, Scott and White Hospital, 2401 South 31st St., Temple, TX 76508. Tel.: (817) 774-4073.

Basic and Advanced Laser Surgery in Gynecology, July 31, August 1, and August 2, 1986, Sheraton Beach Inn, Virginia Beach, Virginia. For more information, please contact: Michael Baggish, M.D., Chief, Department of Ob/Gyn, Crouse Irving Memorial Hospital, 736 Irving Ave., Syracuse, NY 13210 Tel.: (315) 470-7903.

Eighth Practical Course in Colposcopy and Cervico-Vaginal Histopathology (in English), Paris, France, September 22-26, 1986. For further information contact: Dr. R. Cartier, 20 Rue Des Cordelieres, F75013 Paris, France.

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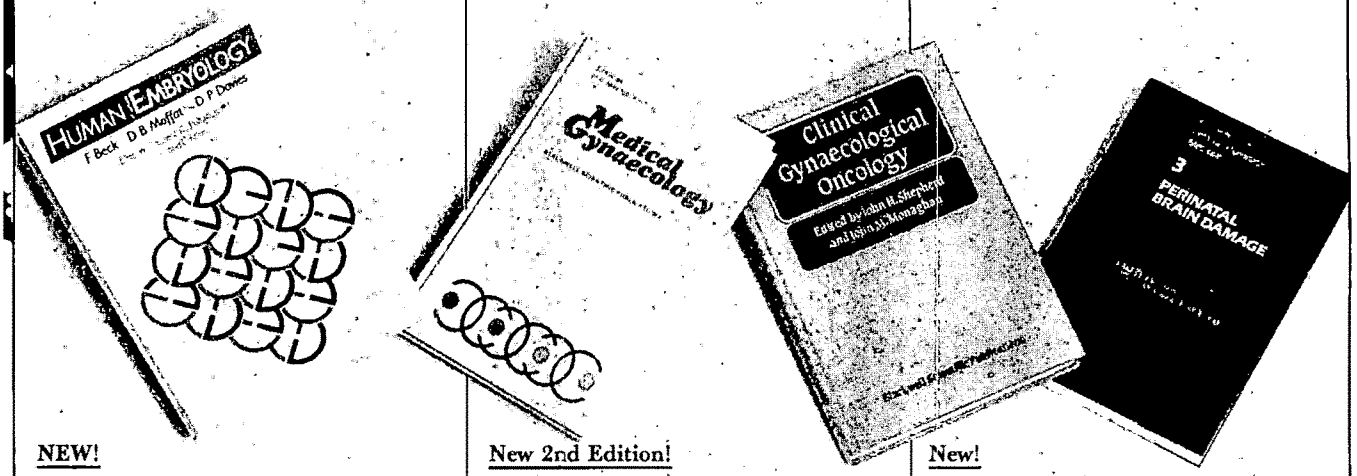
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Ogen 39A,40A,41A

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Tri-Levlen 22A,23A,24A

Carle Clinic
Opportunity Available 58A

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Opportunity Available 10A

Cigna Healthplan of Arizona
Opportunity Available 59A

Churchill Livingstone, Inc.
Books 42A

College of Physicians & Surgeons/Columbia University
Conference 58A

Fairview Hospital
Opportunity Available 58A

FHP Professional Staffing
Opportunity Available 59A

Group Health Plan
Opportunity Available 10A

Highline Community College
Opportunity Available 59A

Indiana University School of Medicine
Opportunity Available 60A

Johns Hopkins Medical Institutions
Course 62A

Kaiser Permanente
Opportunity Available 46A

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Books 53A

Marshfield Clinic
Opportunity Available 46A

Mead Johnson Laboratories
Estrace 54A,55A,56A
Natalins 16A

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Opportunity Available 42A

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HemoCue 12A,13A

Merck Sharp & Dohme
Mefoxin 44A,45A,46A

Miles Pharmaceuticals
Mycelex-G 8A,9A,10A

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Books 63A

Ohio State University
Opportunity Available 61A

Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 .. Third Cover,Fourth Cover

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Opportunity Available 62A

Purdue Frederick Company
Betadine 6A

Roxbury Comprehensive Community Health Center, Inc.
Opportunity Available 61A

Saint John Hospital
Opportunity Available 61A

San Joaquin General Hospital
Opportunity Available 51A

Smith Kline & Beckman
Cefizox 18A,19A

Sonicaid Inc.
FM5 Antepartum Monitor 51A

Southern Colorado Clinic
Opportunity Available 62A

Syntex Laboratories, Inc.
Femstat 47A,48A,49A,50A
Tri-Norinyl ..... Second Cover,1A,2A

Ultrasonix
Doppler 43A

University of Illinois College of Medicine
Opportunity Available 62A

University of Louisville School of Medicine
Opportunity Available 51A

Upjohn Company
Provera 3A,4A

U. S. Air Force
Recruitment 30A,31A

VLI Corporation
Today Sponge 32A

Wyeth Laboratories
Triphasil 33A,34A,35A,36A,37A,38A

Yale University Health Services
Opportunity Available 61A

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing 0.05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

ORTHO-PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

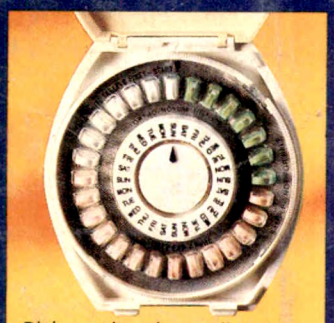
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Please see complete Prescribing Information, a summary of which appears on the preceding page.

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Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

1. 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. BTB comparisons are based on the first three cycles of use. Data available from Syntex Laboratories, Inc.
2. Wynn V, Niththyananthan R: The effect of progestins in combined oral contraceptives on serum lipids with special reference to high-density lipoproteins. *Am J Obstet Gynecol* 142:766-772, 1982.
3. Wynn V: Effect of duration of low-dose oral contraceptive administration on carbohydrate metabolism. *Am J Obstet Gynecol* 142:739-746, 1982.
4. In an independent survey the Wallette™ pill dispenser was preferred to the Ortho Dialpak by 7 out of 10 prospective OC patients. Data available from Syntex Laboratories, Inc.

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TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
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ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM:* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.
MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) then among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences,* or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned.
Precautions *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings.
Adverse Reactions An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms, (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

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PALO ALTO, CA 94304

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching.Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

Caution: Federal law prohibits dispensing without prescription.

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A Standard



Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

A Standard in Progesterone Therapy

Please see adjacent page for brief summary of prescribing information.

MOSBY

American Journal of Obstetrics and Gynecology

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Contents

June
1986

This issue contains Transactions of the Fifty-second Annual Meeting of the Pacific Coast Obstetrical and Gynecological Society and Transactions of the Fifty-third Annual Meeting of The Central Association of Obstetricians and Gynecologists, beginning on page 1285.

TRANSACTIONS OF THE FIFTY-SECOND ANNUAL MEETING OF THE PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY

Your morality or mine? A inquiry into the ethics of human reproduction 1173
Edward C. Hill, M.D.
San Francisco, California

Estrogen use and cardiovascular disease 1181
Brian E. Henderson, M.D., Ronald K. Ross, M.D., Annlia Paganini-Hill, Ph.D., and Thomas M. Mack, M.D.
Los Angeles, California

Fetal injury prior to labor: Does it happen? 1187
Richard H. Paul, M.D., M. Lynn Yonekura, M.D., Cathy Jo Cantrell, M.D., Susan Turkel, M.D., Zdena Pavlova, M.D., and Louise Sipos, R.N., M.Ed.
Los Angeles, California

(Contents continued on page 7A)

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Contents continued from page 5A

Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus 1194
Charles B. Chambers, M.D., and Joseph S. Unis, M.D.
Pomona, California

Partial colpocleisis 1200
Charles F. Langmade, M.D., and Joseph A. Oliver, Jr., M.D.
Pasadena, California

A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance 1206
Douglas Der Yuen, M.D.
Seattle, Washington

Modified retropubic cystourethropexy 1211
John A. Goodno, Jr., M.D., and Thomas W. Powers, M.D.
Arcadia, California

Tubal conservation in ectopic pregnancy: A study of 200 cases 1216
Jack G. Hallatt, M.D.
Los Angeles, California

Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention 1222
William M. Petty, M.D., Richard O. Lowy, M.D., and Albert A. Oyama, M.D.
Portland, Oregon

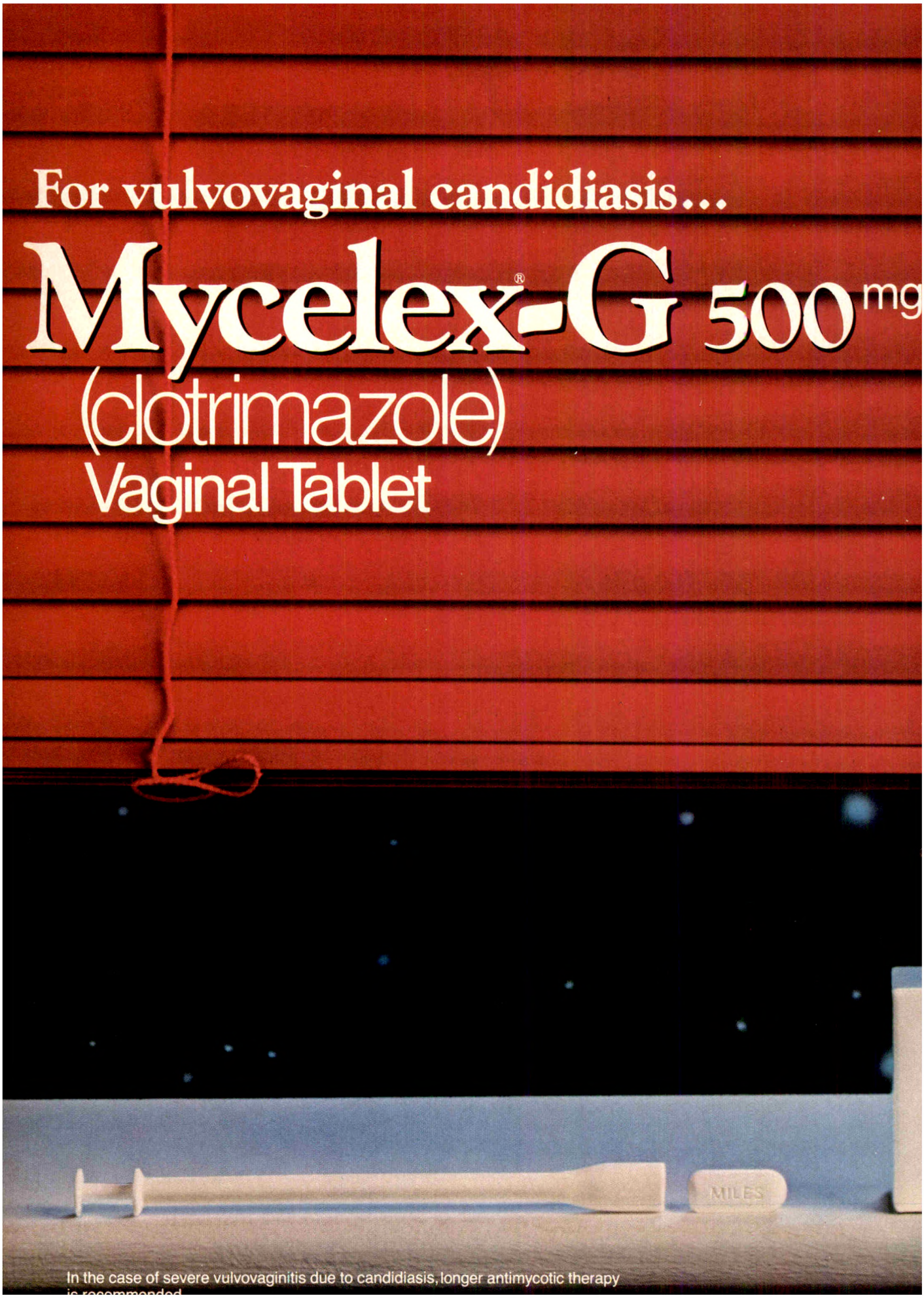
Expanded toxemia syndrome or gestosis 1227
Robert C. Goodlin, M.D.
Denver, Colorado, and Omaha, Nebraska

Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis 1234
Elinor M. Zorn, M.D., Frederick W. Hanson, M.D., L. Carl Greve, Ph.D.,
Barbara Phelps-Sandall, M.S., and Frances R. Tennant, Ph.D.
Davis, California

Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist 1241
Kenneth A. Steingold, M.D., Howard L. Judd, M.D., Roberta K. Nieberg, M.D.,
John K. H. Lu, Ph.D., and R. Jeffrey Chang, M.D.
Los Angeles, California

Chorionic villus sampling: Experience of the first 1000 cases 1249
W. A. Hogge, M.D., S. A. Schonberg, M.D., and M. S. Golbus, M.D.
San Francisco, California

(Contents continued on page 11A)


For vulvovaginal candidiasis...
Mycelex®-G 500 mg
(clotrimazole)
Vaginal Tablet
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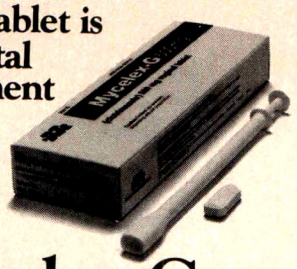
See the following page for prescribing information.

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One tablet is the total treatment



Mycelex®-G 500 mg

(clotrimazole) Vaginal Tablet

INDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are indicated for the local treatment of vulvovaginal candidiasis when one day therapy is felt warranted. In the case of severe vulvovaginitis due to candidiasis, longer antimycotic therapy is recommended. The diagnosis should be confirmed by KOH smears and/or cultures. Other pathogens commonly associated with vulvovaginitis, *Trichomonas* and *Gardnerella (Haemophilus) vaginalis*, should be ruled out by appropriate laboratory methods.

CONTRAINDICATIONS: Mycelex®-G 500 mg Vaginal Tablets are contraindicated in women who have shown hypersensitivity to any components of the preparation.

WARNINGS: None.

PRECAUTIONS: If there is a lack of response to Mycelex®-G 500 mg Vaginal Tablets, appropriate microbiological studies should be repeated to confirm the diagnosis and rule out other pathogens before instituting another course of antimycotic therapy.

CARCINOGENESIS: No long term studies in animals have been performed to evaluate the carcinogenic potential of Mycelex®-G 500 mg Vaginal Tablets intravaginally. A long term study in rats (Wistar strains) where clotrimazole was administered orally provided no indication of carcinogenicity.

USAGE IN PREGNANCY: PREGNANCY CATEGORY B: The disposition of ^{14}C-clotrimazole has been studied in humans and animals. Clotrimazole is poorly absorbed following intravaginal administration to humans, whereas it is rather well absorbed after oral administration.

In clinical trials, use of vaginally applied clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well-controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats given repeated intravaginal doses up to 100 mg/kg/day have revealed no evidence of harm to the fetus due to clotrimazole.

Repeated high oral doses of clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose.

Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

ADVERSE REACTIONS: Of 297 patients in double-blind studies with the 500 mg vaginal tablet, 3 of 149 patients treated with active drug and 3 of 148 patients treated with placebo reported complaints during therapy that were possibly drug related. In the active drug group, vomiting occurred in one patient, vaginal soreness with coitus in another, and complaints of vaginal irritation, itching, burning and dyspareunia in the third patient. In the placebo group, clitoral irritation occurred in one patient and dysuria, described as remotely related to drug, in the other. A third patient in the placebo group developed bacterial vaginitis which the investigator classed as possibly related to drug.

Eighteen (1.6%) of the 1116 patients treated with Mycelex®-G in other formulations in double-blind studies reported complaints during therapy that were possibly drug-related. Mild burning occurred in six patients while other complaints such as skin rash, itching, vulval irritation, lower abdominal cramps and bloating, slight cramping, slight urinary frequency, and burning or irritation in the sexual partner, occurred rarely.

OVERDOSAGE: No data available.

DRUG ABUSE AND DEPENDENCE: Drug abuse and dependence with Mycelex®-G 500 mg Vaginal Tablets has not been reported.

DOSAGE AND ADMINISTRATION: The recommended dose is one tablet inserted intravaginally one time only, preferably at bedtime. In the event of treatment failure, that is, persistence of signs and symptoms of vaginitis after five days, other pathogens commonly responsible for vaginitis should be ruled out before instituting another course of antimycotic therapy.

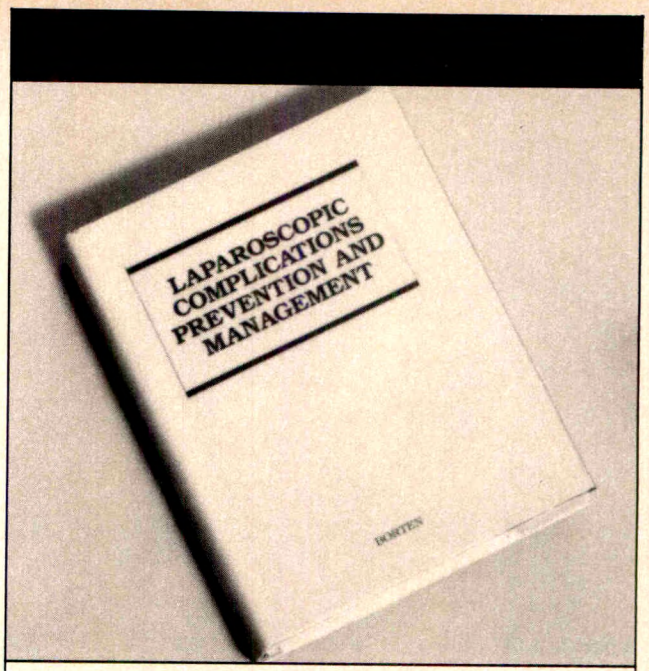
HOW SUPPLIED: Mycelex®-G 500 mg Vaginal Tablets are white, bullet shaped, uncoated tablets, coded with Miles on one side and 097 on the other, supplied as a single 500 mg tablet with plastic applicator and patient instructions.

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Contents continued from page 7A

Detection of preterm labor by ambulatory monitoring of uterine activity for the management of oral tocolysis 1253
Michael Katz, M.D., Pamela J. Gill, R.N., M.S.N., and Roger B. Newman, M.D.
San Francisco, California

The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman 1257
Deborah A. Ray, M.D., John D. Yeast, M.D., and Roger K. Freeman, M.D.
Long Beach and Orange, California

Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization 1264
Karl Luber, M.D., Charles C. Beeson, M.D., Joseph F. Kennedy, M.D., Benito Villanueva, M.D., and Philip E. Young, M.D.
San Diego, California

Fetomaternal hemorrhage associated with umbilical vein thrombosis 1271
Roger W. Hoag, M.D.
Berkeley and San Francisco, California

Preinduction cervical ripening with sequential use of prostaglandin E_2 gel 1275
Robert P. Prins, M.D., Duncan R. Neilson, Jr., M.D., Richard N. Bolton, M.D., Carl Mark III, M.D., and Peter Watson, M.D.
Portland, Oregon

On the origin and progression of human breast cancer 1280
Hanne M. Jensen, M.D.
Davis, California

TRANSACTIONS OF THE FIFTY-THIRD ANNUAL MEETING OF THE CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

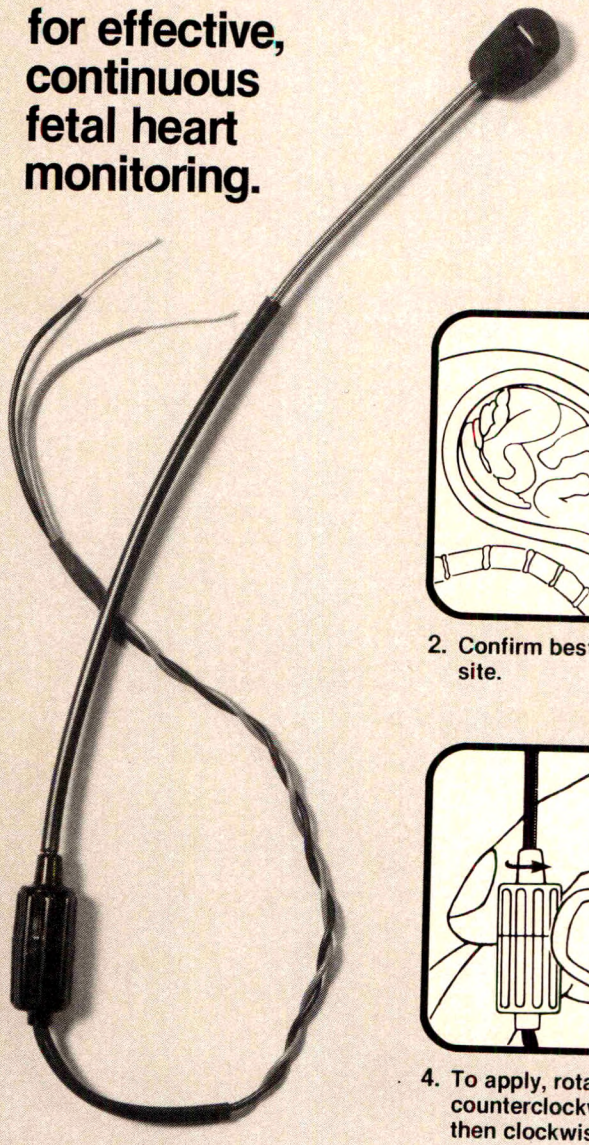
The Golden Thread 1285
George D. Malkasian, Jr., M.D.
Rochester, Minneosta

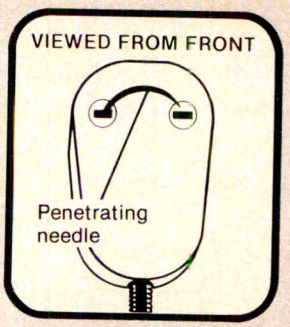
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay 1290
K. J. Chacho, M.D., M. Stronkowski Chacho, M.D., P. J. Andresen, B.S., and A. Scommegna, M.D.
Chicago, Illinois

(Contents continued on page 13A)

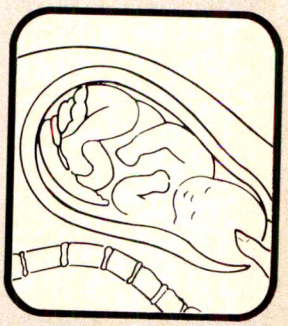
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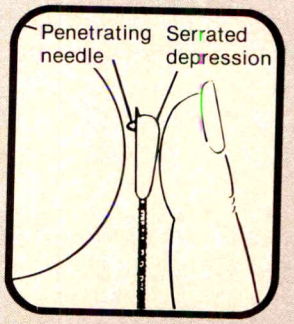




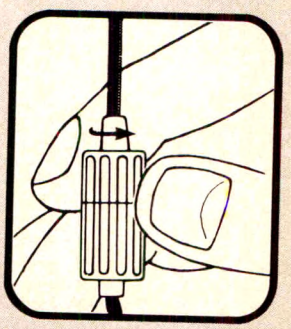
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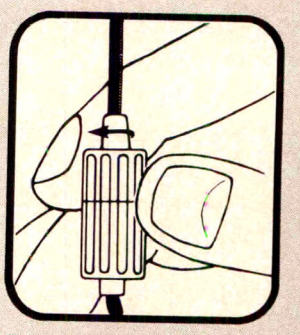
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Contents continued from page 11A

Conservative treatment of ectopic pregnancy with methotrexate 1299
Steven J. Ory, M.D., Alelei L. Villanueva, M.D., Peter K. Sand, M.D.,
and Ralph K. Tamura, M.D.
Chicago, Illinois

Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings 1306
Frank O. Page, M.D., James N. Martin, M.D., Sue M. Palmer, M.D.,
Rick W. Martin, M.D., John A. Lucas, M.D., G. Rodney Meeks, M.D.,
Edsel T. Bucovaz, Ph.D., and John C. Morrison, M.D.
Jackson, Mississippi

Upper genital tract changes and infertility in diethylstilbestrol-exposed women 1312
Raymond H. Kaufman, M.D., Ervin Adams, M.D., Kenneth Noller, M.D.,
John F. Irwin, M.D., and Milton Gray, M.D.
Houston, Texas, and Rochester, Minnesota

Primary carcinoma of the fallopian tube 1319
Karl C. Podratz, M.D., Ph.D., Edward S. Podczaski, M.D., Thomas A. Gaffey, M.D.,
Peter C. O'Brien, Ph.D., Mark F. Schray, M.D., and George D. Malkasian, Jr., M.D.
Rochester, Minnesota

Immunoglobulin G subclasses and isoimmunized pregnancy outcome 1327
M. Mark Taslimi, M.D., Baha M. Sibai, M.D., James M. Mason, Ph.D., and
John V. Dacus, M.D.
Memphis, Tennessee

Experience with intrauterine insemination for cervical factor and oligospermia 1333
Magdalen E. Hull, M.D., David M. Magyar, D.O., Jaime M. Vasquez, M.D.,
Maria F. Hayes, M.D., and Kamran S. Moghissi, M.D.
Detroit, Michigan

An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears 1339
David W. Wetrich, M.D.
Iowa City, Iowa

Chorionic villus sampling in continuing pregnancies. I. Low fetal loss rates in initial 109 cases 1349
Sherman Elias, M.D., Joe Leigh Simpson, M.D., Alice O. Martin, Ph.D.,
Rudy Sabbagha, M.D., Allan Bombard, M.D., Barbara J. Rosinsky, B.S.,
and Lora D. Baum, M.S.
Chicago, Illinois

(Contents continued on page 16A)

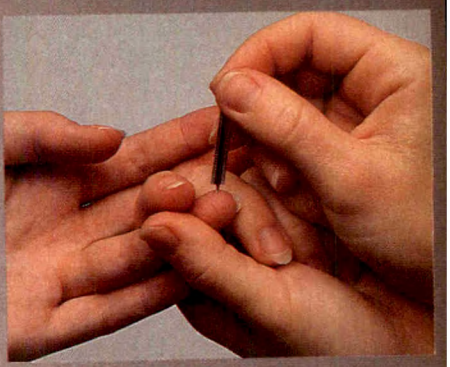
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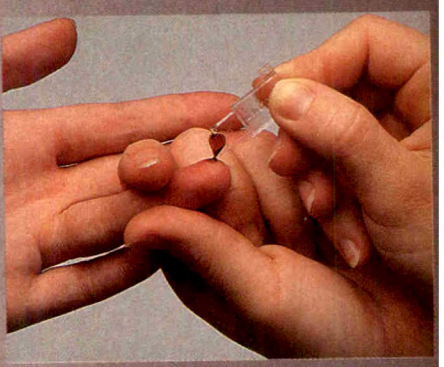


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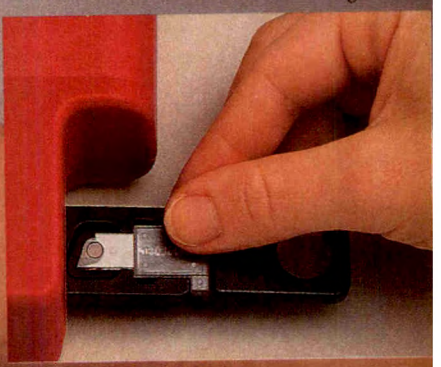
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Contents continued from page 13A

Chorionic villus sampling in continuing pregnancies. II. Cytogenetic reliability 1353
Alice O. Martin, Ph.D., Joe Leigh Simpson, M.D., Barbara J. Rosinsky, B.S., and Sherman Elias, M.D.
Chicago, Illinois

Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women 1363
Charles M. Stedman, M.D., Judy C. Baudin, M.T.(A.S.C.P.), S.B.B., Charles A. White, M.D., and E. Shannon Cooper, M.D.
New Orleans, Louisiana

BOOKS

Books received 1370

ITEMS

Items 1371

INDEX

Author index 1395

Subject index 1409

Information for authors on pages 25A, 26A, and 27A

Index to advertisers on page 52A

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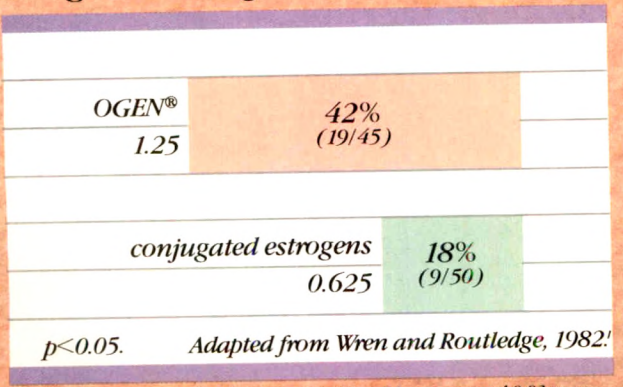
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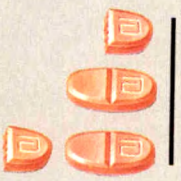
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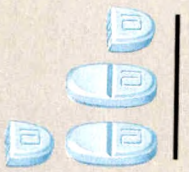
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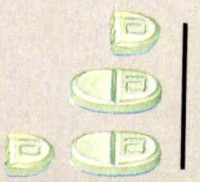
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1. Wren BG, Brown LB, Routledge DA: Differential clinical response to oestrogens after menopause. *Med J Aust* 2:329-332, 1982.

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WARNING:

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. OGEN SHOULD NOT BE USED DURING PREGNANCY.

According to some investigators, the use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. Studies have reported that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. In one of these studies, this risk was estimated as not greater than 4 per 1000 exposures. Furthermore, there are reports that a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these reported changes are histologically benign, the investigators have not determined whether they are precursors of adenocarcinoma.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies in the offspring, including heart defects and limb reduction defects. One case control study estimated a 4.7 fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. OGEN has not been studied for these uses, and therefore should not be used during pregnancy. There is no evidence from well controlled studies that progestogens are effective for these uses.

If OGEN (estropipate tablets) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the question of continuation of the pregnancy should be addressed.

INDICATIONS AND USAGE

The cyclic administration (See "DOSAGE AND ADMINISTRATION" section) of OGEN (estropipate tablets) is indicated for the treatment of estrogen deficiency associated with:

1. Moderate to severe *vasomotor* symptoms of menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.

OGEN (ESTROPIPATE TABLETS) HAS NOT BEEN TESTED FOR EFFICACY FOR ANY PURPOSE DURING PREGNANCY. SINCE ITS EFFECT UPON THE FETUS IS UNKNOWN, IT CANNOT BE RECOMMENDED FOR ANY CONDITION DURING PREGNANCY (SEE BOXED WARNING).

CONTRAINDICATIONS

OGEN should not be used in women with any of the following conditions:

1. Known or suspected cancer of the breast.
2. Known or suspected estrogen-dependent neoplasia.
3. OGEN may cause fetal harm when administered to a pregnant woman. OGEN is contraindicated in women who are or may become pregnant (See Boxed Warning).
4. Undiagnosed abnormal genital bleeding.
5. Active thrombophlebitis or thromboembolic disorders.
6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use.

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species has been reported by some investigators to increase the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning).

At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. Therefore, caution should be exercised when administering estrogens to women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. Careful breast examinations should be performed periodically.

2. *Gall bladder disease.* A recent study has reported a 2 to 3-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of oral contraceptives. In the case of oral contraceptives, the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects of oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrogen therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expected that the larger doses of estrogen used to treat postpartum breast engorgement would be more likely to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in women receiving estrogens for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of various thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism; it should also be discontinued during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not been found this does not rule out the possibility that such an increase may be present or that subgroups of women who have underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be used in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Although benign, and rare, these may rupture and cause death through intraabdominal hemorrhage. Such lesions have not yet been reported in association with other estrogen or progestogen preparations but should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also been reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is not known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now a report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receiving estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

PRECAUTIONS

A. General Precautions.

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed.

2. Fluid retention — Estrogens may cause some degree of fluid retention. Therefore, patients with conditions such as epilepsy, migraine, and cardiac or renal dysfunction, which might be influenced by this factor, require careful observation.

3. Certain patients may develop undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not clear whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depression should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of the patient's use of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered with caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patients with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

B. Information for the Patient. See text of Patient Package Insert which appears after PHYSICIAN REFERENCES.

C. Drug Interactions. The concomitant use of any drugs which can induce hepatic microsomal enzymes with estrogens may produce estrogen levels which are lower than would be expected from the dose of estrogen administered.

The use of *broad spectrum antibiotics* which profoundly effect intestinal flora may influence the absorption of steroidal compounds including the estrogens.

Diabetics receiving *insulin* may have increased insulin requirements when receiving estrogens.

Laboratory Test Interference. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The following similar changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Abnormal glucose tolerance test results.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

D. Carcinogenesis. Studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods (see Boxed Warning). At the present time there is no conclusive evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast. There are, however, a few retrospective studies which suggest a small but statistically significant increase in the risk factor for breast cancer among these women. (See "WARNINGS" section.)

E. Pregnancy. Pregnancy Category X. See "CONTRAINDICATIONS" section and Boxed Warning.

F. Nursing Mothers. Estrogens have been reported to be excreted in human breast milk. Caution should be exercised when OGEN is administered to a nursing woman.

G. Pediatric Use. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in whom bone growth is not complete.

ADVERSE REACTIONS

(See Warnings regarding reports of possible induction of neoplasia, unknown effects upon the fetus, increased incidence of gall bladder disease, and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adverse reactions in decreasing order of severity within each category have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system.*
Increase in size of uterine fibromyomata.
Vaginal candidiasis.
Cystitis-like syndrome.
Dysmenorrhea.
Amenorrhea during and after treatment.
Change in cervical eversion and in degree of cervical secretion.
Breakthrough bleeding, spotting, change in menstrual flow.
Premenstrual-like syndrome.
2. *Breast.*
Tenderness, enlargement, secretion.
3. *Gastrointestinal.*
Cholestatic jaundice.
Vomiting, nausea.
Abdominal cramps, bloating.
4. *Skin.*
Hemorrhagic eruption.
Erythema nodosum.
Erythema multiforme.
Hirsutism.
Chloasma or melasma which may persist when drug is discontinued.
Loss of scalp hair.
5. *Eyes.*
Steepening of corneal curvature.
Intolerance to contact lenses.
6. *CNS.*
Chorea.
Mental depression.
Migraine, dizziness, headache.
7. *Miscellaneous.*
Aggravation of porphyria.
Edema.
Reduced carbohydrate tolerance.
Increase or decrease in weight.
Changes in libido.

OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate that serious ill effects do not occur. Overdosage of estrogen may cause nausea and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION

1. *Given cyclically for short-term use:*

For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (e.g., 3 weeks on and 1 week off).

Attempts to discontinue or taper medication should be made at 3 to 6 month intervals.

Usual dosage ranges:

Vasomotor symptoms —One OGEN .625 Tablet to one OGEN 5 Tablet per day. The lowest dose that will control symptoms should be chosen. If the patient has not menstruated within the last two months or more, cyclic administration is started arbitrarily. If the patient is menstruating, cyclic administration is started on day 5 of bleeding.

Atrophic vaginitis and kraurosis vulvae — One OGEN .625 Tablet to one OGEN 5 Tablet daily, depending upon the tissue response of the individual patient. The lowest dose that will control symptoms should be chosen. Administer cyclically.

2. *Given cyclically:*

Female hypogonadism; female castration; primary ovarian failure.

Usual dosage ranges:

Female hypogonadism — A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. The lowest dose that will control symptoms should be chosen. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium. If satisfactory withdrawal bleeding does not occur, an oral progestogen may be given in addition to estrogen during the third week of the cycle.

Female castration and primary ovarian failure —A daily dose of one OGEN 1.25 Tablet to three OGEN 2.5 Tablets may be given for the first three weeks of a theoretical cycle, followed by a rest period of eight to ten days. Adjust dosage upward or downward according to severity of symptoms and response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED

OGEN (estropipate tablets, USP) is supplied as OGEN .625 (0.75 mg estropipate), yellow tablets, **NDC** 0074-3943-04; OGEN 1.25 (1.5 mg estropipate), peach-colored tablets, **NDC** 0074-3946-04; OGEN 2.5 (3 mg estropipate), blue tablets, **NDC** 0074-3951-04; and OGEN 5 (6 mg estropipate), light green tablets, **NDC** 0074-3958-13. Tablets of all four dosage levels are standardized to provide uniform estrone activity and are grooved (Divide-Tab®) to provide dosage flexibility. All tablet sizes of OGEN are available in bottles of 100.

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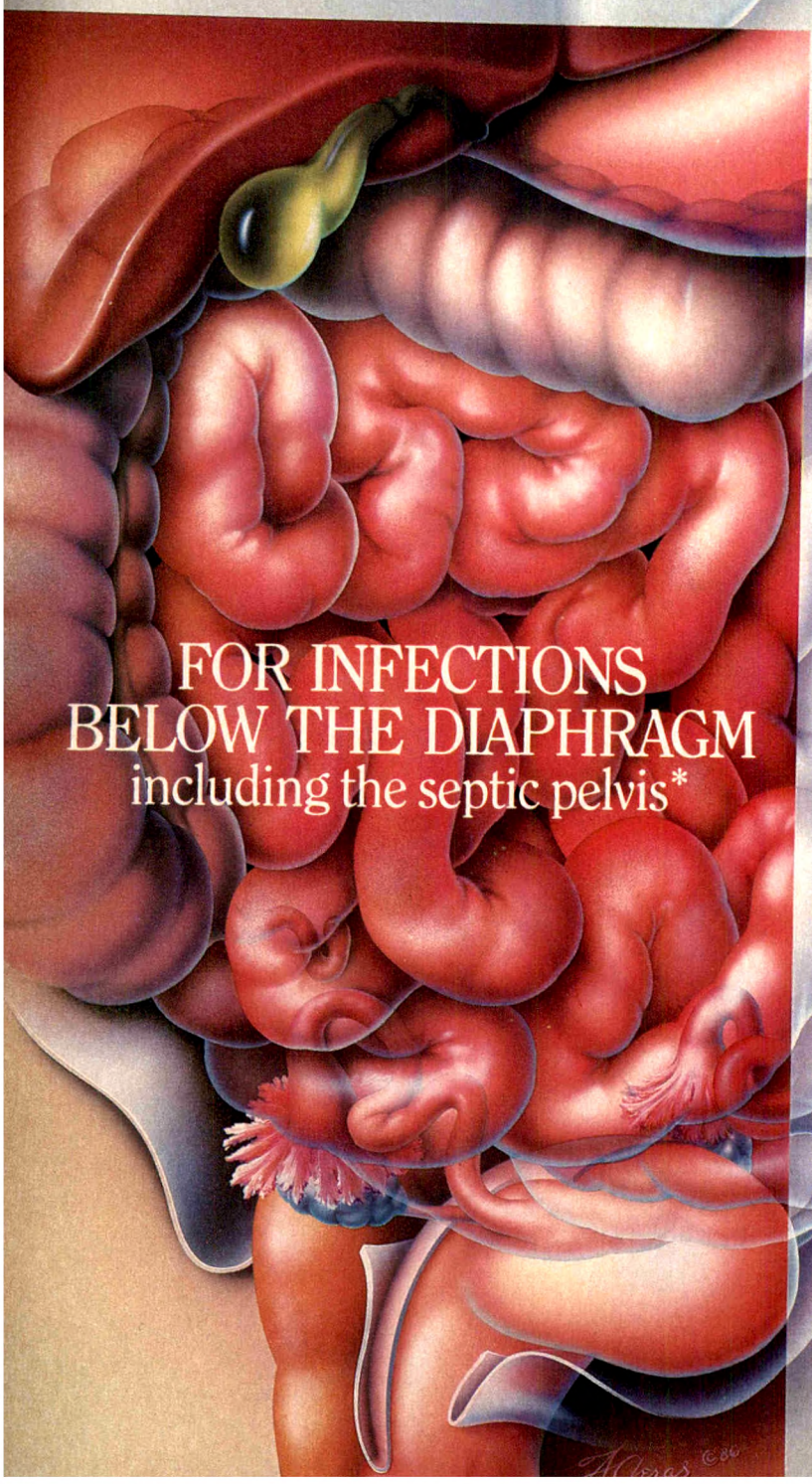
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A recent, prospective, randomized, comparative trial demonstrated that 'Cefizox' was equal to clindamycin/tobramycin in the treatment of intra-abdominal infections.

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Aerobic Organisms[2] (number of strains)	CEFIZOX® (ceftizoxime) MIC_{90}	MEFOXIN® (cefoxitin) MIC_{90}
Gram-Negative	**mcg./ml.**	**mcg./ml.**
Escherichia coli (40)	0.04	6.3
Klebsiella pneumoniae (32)	0.05	25
Enterobacter aerogenes (16)	0.12	>400
Enterobacter cloacae (16)	0.8	400
Proteus sp. (indole positive) (26)	6.3	12.5
Citrobacter sp. (32)	0.2	400
Gram-Positive	**mcg./ml.**	**mcg./ml.**
Staphylococcus aureus (16)	3.1	3.1

Bacteroides species[3] (number of strains)	CEFIZOX® (ceftizoxime) MIC_{90}	MEFOXIN® (cefoxitin) MIC_{90}
B. fragilis (55)	8 mcg./ml.	16 mcg./ml.
B. ovatus (17)	16 mcg./ml.	64 mcg./ml.
B. thetaiotaomicron (13)	64 mcg./ml.	32 mcg./ml.
B. distasonis (12)	8 mcg./ml.	32 mcg./ml.
B. vulgatus (3)	8 mcg./ml.	16 mcg./ml.

* Due to susceptible strains of indicated organisms.
† Dosage in renally compromised patients may require adjustment. Please see complete prescribing information.
‡ *In vitro* activity does not necessarily imply *in vivo* effectiveness.

1. Harding, G.K.M., et al.: Rev. Infect. Dis. 6(Suppl. 1) S283-S292, 1984.
2. Fu, K.P., and Neu, H.C.: Antimicrob. Agents Chemother. 17:583-590, 1980.
3. Aldridge, K.E., et al.: Antimicrob. Agents Chemother. 26:410-413, 1984.

Please consult last page of this advertisement for brief summary of prescribing information.

Cefizox®

brand of

sterile ceftizoxime sodium and ceftizoxime sodium injection

Before prescribing, see complete prescribing information in SK&F literature or *PDR*. The following is a brief summary.

INDICATIONS

'Cefizox' is indicated in the treatment of infections due to susceptible strains of the microorganisms listed below:

LOWER RESPIRATORY TRACT INFECTIONS caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Klebsiella* sp.; *Proteus mirabilis; Escherichia coli; Haemophilus influenzae* including ampicillin-resistant strains; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Serratia* sp.; *Enterobacter* sp.; and *Bacteroides* sp.

URINARY TRACT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Pseudomonas* sp. including *Ps. aeruginosa; Proteus mirabilis; P. vulgaris; Providencia rettgeri* (formerly *Proteus rettgeri*) and *Morganella morganii* (formerly *Proteus morganii*); *Klebsiella* sp.; *Serratia* sp. including *S. marcescens; Enterobacter* sp.

GONORRHEA. Uncomplicated cervical and urethral gonorrhea caused by *Neisseria gonorrhoeae.*

INTRA-ABDOMINAL INFECTIONS caused by *Escherichia coli; Staphylococcus epidermidis; Streptococcus* sp. (excluding enterococci); *Enterobacter* sp.; *Klebsiella* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

SEPTICEMIA caused by *Streptococcus* sp. including *S. pneumoniae* (formerly *D. pneumoniae*), but excluding enterococci; *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Escherichia coli; Bacteroides* sp. including *B. fragilis; Klebsiella* sp.; and *Serratia* sp.

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Staphylococcus epidermidis; Escherichia coli; Klebsiella* sp.; *Streptococcus* sp. including *Streptococcus pyogenes* (group A beta-hemolytic), but excluding enterococci; *Proteus mirabilis; Serratia* sp.; *Enterobacter* sp.; *Bacteroides* sp. including *B. fragilis;* and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

BONE AND JOINT INFECTIONS caused by *Staphylococcus aureus* (penicillinase and nonpenicillinase producing); *Streptococcus* sp. (excluding enterococci); *Proteus mirabilis; Bacteroides* sp.; and anaerobic cocci, including *Peptococcus* sp. and *Peptostreptococcus* sp.

MENINGITIS caused by *Haemophilus influenzae*. 'Cefizox' has also been used successfully in the treatment of a limited number of pediatric and adult cases of meningitis caused by *Streptococcus pneumoniae.*

'Cefizox' has been effective in the treatment of seriously ill, compromised patients, including those who were debilitated, immunosuppressed or neutropenic.

Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS

'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS

BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

***Pseudomembranous colitis* has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who developed diarrhea in association with antibiotic use.** Treatment with broad-spectrum antibiotics alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile.* Other causes of colitis should also be considered.

PRECAUTIONS

General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal status should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Careful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox,' nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been established.

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should be exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not been established. In children six months of age and older, treatment with 'Cefizox' has been associated with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phospholkinase). The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS

Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, but less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SGOT, SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient elevations of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic*—Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiting have been reported occasionally. Symptoms of pseudomembranous colitis can appear during or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED

Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 grams of ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxime.

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 grams of ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactured for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

BRS — CF:L14

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Atlas of Fetal Sectional Anatomy

With Ultrasound and Magnetic Resonance Imaging

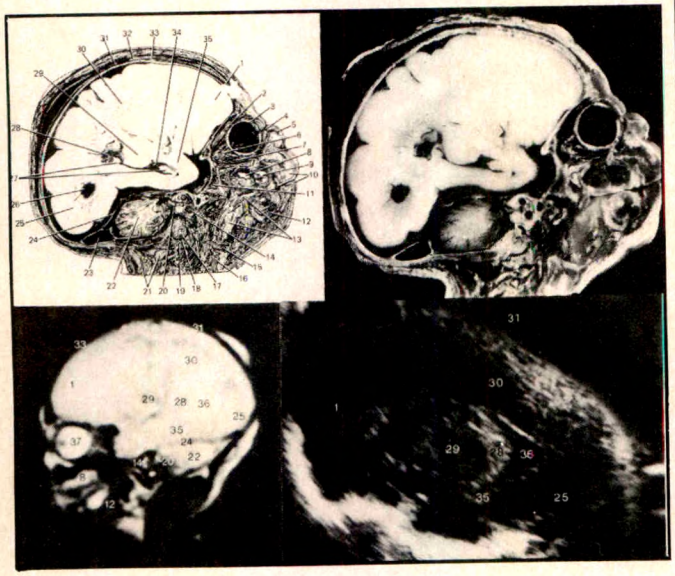
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M. Hansmann, MD; B.-J. Hackelöer, MD; and A. Staudach, MD

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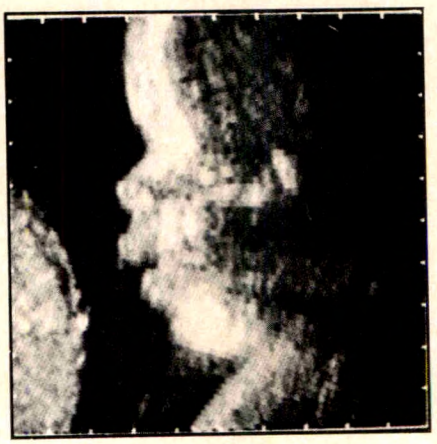


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American Journal of Obstetrics and Gynecology

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INFORMATION FOR AUTHORS

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The requirements for manuscripts submitted to the *American Journal of Obstetrics and Gynecology* conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1982;96(6):766-71. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C. V. Mosby Company or the Editors on request.

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The original and two good-quality copies of the manu-

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

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of each table and each column precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Illustrations and tables should supplement, not duplicate, the text; presentation in either one or the other will suffice.

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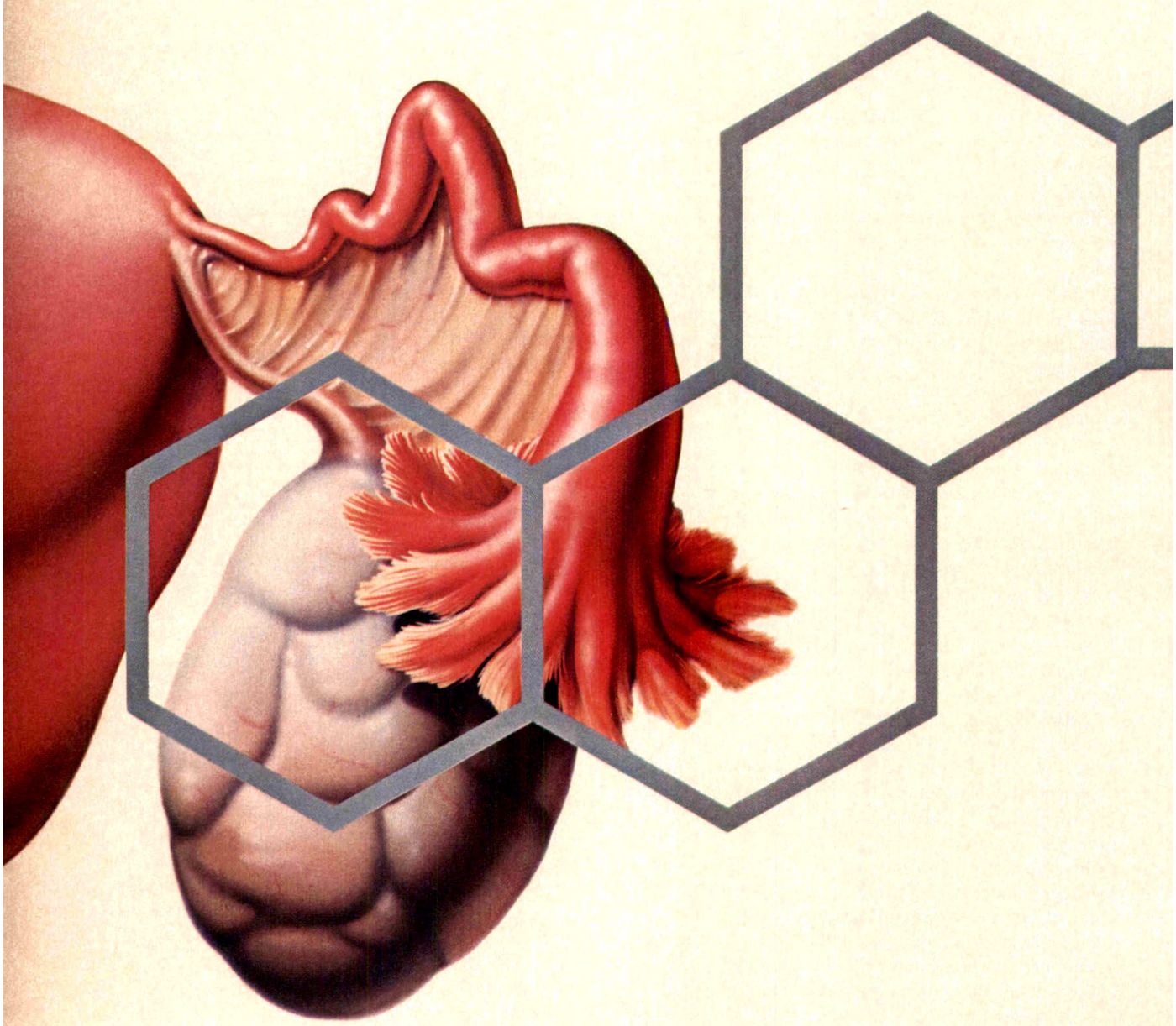
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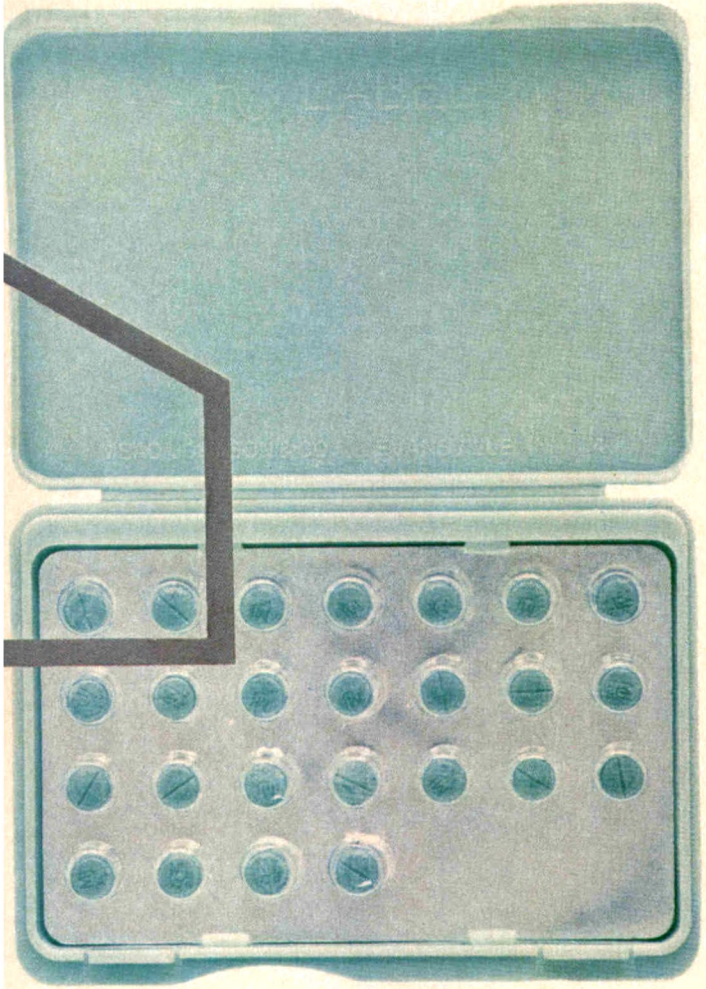
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2. **ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.** The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects. One case control study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed *in utero* to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If ESTRACE® (estradiol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation.

Indications: *Tablets:* Moderate to severe *vasomotor* symptoms associated with the menopause (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.); atrophic vaginitis; kraurosis vulvae; female hypogonadism; female castration; primary ovarian failure; breast cancer (for palliation only) in appropriately selected women and men with metastatic disease; prostatic carcinoma—palliative therapy of advanced disease. *Vaginal Cream:* Atrophic vaginitis and kraurosis vulvae. ESTRACE® HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

Contraindications: Known or suspected cancer of the breast, except in appropriately selected patients being treated for metastatic disease; known or suspected estrogen-dependent neoplasia; known or suspected pregnancy (See Boxed Warning); undiagnosed abnormal genital bleeding; active thrombophlebitis or thromboembolic disorders; a past history of thrombophlebitis, thrombosis or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

Warnings: Estrogens increase the risk of carcinoma of the endometrium (See Boxed Warning). Prescribe cautiously in women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms. A 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease has been reported in women receiving postmenopausal estrogens. There is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis or thromboembolic disorders or (except in treatment of malignancy) with a history of such disorders in association with estrogen use; they should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in whom estrogens are clearly needed. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis; when estrogen doses of this size are used, any of the thromboembolic and thrombotic adverse effects associated with oral contraceptives should be considered a clear risk. Hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Increased blood pressure occurs with use of estrogens in the menopause, and blood pressure should be monitored, especially with high doses. Diabetic patients should be carefully observed for decreased glucose tolerance. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases; if this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium level.

Precautions: *General*—A complete medical and family history should be taken prior to initiation of therapy. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination. Conditions influenced by fluid retention, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Undesirable manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc, may develop. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. Pathologists should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops, discontinue use while cause is investigated. Administer with caution in patients with impaired liver function, metabolic bone diseases associated with hypercalcemia, or renal insufficiency and in young patients with incomplete bone growth. The following endocrine and liver function tests may be affected by larger estrogen doses: increased sulfobromophthalein retention; increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability; increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay; free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered; impaired glucose tolerance; decreased pregnanediol excretion; reduced response to metyrapone test; reduced serum folate concentration; increased serum triglyceride and phospholipid concentration. *Information for the Patient*—See text of Patient Package Insert. *Pregnancy Category X*—See Contraindications and Boxed Warning. *Nursing Mothers*—Administer to nursing mothers only when clearly necessary.

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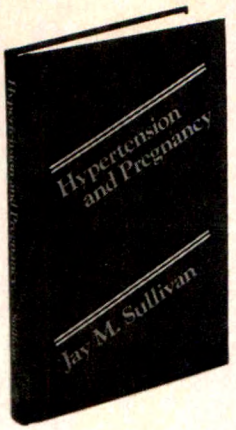
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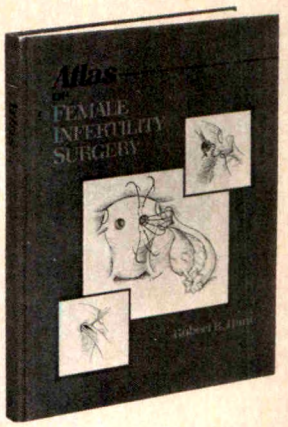
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Edward C. Hill, M.D.

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There are two parts to the human dilemma.
One is the belief that the end justifies the means, . . .
the other is the betrayal of the human spirit:
the assertion of dogma that closes the mind.
Jacob Bronowski[1]
The Ascent of Man

If ethics is defined as the study of ideal conduct, then medical ethics becomes the study of ideal conduct by health care professionals: that is, whether our actions in the pursuit of our profession are right or wrong. To those of us who were graduated from medical school 40 years ago, the term "medical ethics" conjures up images of the Hippocratic oath, with its resolve to do no harm and emphasis on the responsibility of the physician as a teacher. Or perhaps it brings to mind the *Principles of Medical Ethics of the American Medical Association*, the preamble of which stresses the primary benefit of the patient but recognizes the physician's responsibility to society, to other health professionals, and to one's self. In any event, there was no formal training in moral philosophy in medical school. Whatever skill we developed in coping with ethical dilemmas came about as a result of bedside teaching in the Osler tradition. It developed from emulation of those whose value judgments we admired. However, 30 or 40 years ago such value judgments seemingly were relatively simple and straightforward. Families looked to their physicians, as well as their religious leaders, for guidance and counsel. The family physician, as an observer of the human condition in the broadest sense, was at least as well qualified as others to do so. Medical progress had not yet reached the point where technology made it possible to transplant organs, to insert man-made ones, or to sustain indefinitely a life that hung in the balance and where, indeed, even the distinction between life and death could be difficult. The progress of medical science in the past three or four decades has surpassed the wildest dreams of most of its observers and practitioners. But advancing technology has brought with it a host of new problems, and most of these problems have centered around the morality of applying or not applying the technology. Karen Quinlan, Baby Fae, Baby Doe, Barney Clark, William Schroeder, William Bartling, and the Frustaci septuplets are cases in point. Increasingly, medical decisions have come into conflict with moral standards of at least an audible section of our pluralistic society. Now the physician finds his actions being carefully and closely scrutinized by a host of people in other disciplines—sociologists, economists, psychologists, political scientists, politicians, theologians, attorneys, and philosophers. A voluminous literature on medical ethics has evolved

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during the past decade, with over 200 articles a month being published. Much of the rhetoric is counterproductive, "some consisting of largely ungrounded assertions . . . and others mere collections of woesome, top-of-the-head remarks by otherwise creditable scholars."[2] Moreover, the criticism doesn't always pertain to medical decisions made by physicians. Here is an item that illustrates the point. It appeared in the *Hastings Center Report*, the publication of the prestigious Institute of Society, Ethics and Life Sciences, and is entitled "The Best Little Doctors' Investment in Winnemucca":

Silvia Binder, a French woman who has operated three brothels in Winnemucca, Nevada, recently put up her business for sale. Prostitution is legal in Winnemucca, a little town of 4,000 residents 150 miles from Reno. However, according to U.S. Naturalization Service regulations, it is a "deportable occupation," and as a noncitizen Mme. Binder faced deportation for her activities. According to a UPI report (*New York Times*, December 17, 1982) a group of California doctors came up with the $2.5 million purchase price. Their background will have to be checked by the police before the license transfer is approved, and if they pass the test, their names will be made public. The investment broker who arranged the deal said that "it makes a lot of sense financially." But is it ethical? That question seems not have come up.[3]

The editor failed to say whether the ethical question was the purchase of the bordello or the publication of the physicians' names.

To be serious, though, there is a comprehensive literature in bioethics that is valid and thought-provoking, offering at least different viewpoints on issues that all of us find troubling. Physicians in general, and obstetrician-gynecologists in particular, have difficulty enough in keeping up with the scientific literature, having little opportunity to familiarize themselves with publications airing issues of medical ethics. It is my purpose to summarize an inquiry into the recent literature on this subject, especially as it applies to obstetrics and gynecology, and to try to formulate a conclusion based on that inquiry.

Certainly, medical ethics is not new, but in the past 10 years it has received a great deal of attention. Almost all medical schools have introduced formal ethics courses, most of them conducted by nonphysicians. Most hospitals now have an ethics committee with a multidisciplinary composition. A Presidential Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, appointed in 1979, recently summarized its findings.[4] With all of this activity, one gains the impression that medical morality must be different from common, everyday morality, but it is not. What makes it notable is that it deals with topics that are highly complex and emotionally charged. It involves situations to which everyone can relate in terms of his or her own health or well-being. Moreover, as one observer[5] has noted, it may be a question in which "the facts, probabilities, distinctions, risks and benefits are so involved that it is not easy to act in accord with moral rules even if you want to." To compound the problem, and the medical profession is in a large measure responsible for doing so, technology has given the impression to the public that it is capable of anything. We have created the illusion that with appropriate tests, diagnoses are not missed, and with proper treatment, cures are ensured. With the availability of pacemakers, coronary bypasses, artificial hearts, and kidney, heart-lung, and liver transplants, eternal life is attainable. If anything goes wrong, it is the fault of the physician. The media, along with a great deal of wishful thinking on the part of everyone, have fostered and nourished this illusion. It has been said[6] that physicians have all too unquestioningly accepted the burden of being healers of all the ills of mankind.

Little is said about the enormous degree of uncertainty that exists in medical practice, a fact we live with daily. We have been accused of not disclosing uncertainty for fear of losing power and control over the decision-making process or losing the confidence of the patient. A major benefit of modern medical technology is a significant reduction in the degree of uncertainty. Remember what it was like to practice without the benefit of sophisticated laboratory tests, sonography, computerized axial tomography scanners, magnetic resonance imaging, mammography, amniocentesis, laparoscopy, fetal heart monitoring, chorionic villus sampling, antibiotics, blood banks, methotrexate, and Medline searches? The quality of medical care has never been better, but the overall confidence of the public in the medical profession has never been worse. In part this is due to the existence and use of modern medical technology oftentimes as a substitute for the development of the traditional doctor-patient relationship. Technology begs to be used! There is no question in my mind that there is rampant overutilization. Another responsible factor is the fragmentation of medical care into the various specialties and subspecialties so that the patient is never treated as a "whole person." In his delightful essay entitled "The Seven Sins of Medicine," Richard Asher[7] listed overspecialization as one of the sins and related that he had seen an ophthalmologist, when asked to see a patient with retinitis pigmentosa, write in the chart, "This might be part of the Laurence-Moon-Biedl syndrome; is there any evidence of polydactyly?"

At any rate, the medical ethics debate has picked up a full head of steam, and because many of the moral issues of the day involve human reproduction, the practice of obstetrics and gynecology is right in the middle

of it. Abortion, adolescent sexuality, artificial insemination, contraception, defective newborns, genetic screening, in utero treatment, in vitro fertilization, sex preselection, and surrogate motherhood all feature prominently in current discussions. These are not just medical issues, however; they are social issues as well and thus are the concern of everyone.

Kenneth Ryan,[8] in his thoughtful Joseph Price Oration at the Third Annual Meeting of the American Gynecological and Obstetrical Society last year, pointed out the fallacy of discussing the morality of sexual attitudes and the control of human reproduction without a knowledge of the historical evolution of these aspects of human behavior. The move toward abortion reform in the State of California and the role played by some of the members of this society are vivid in the memories of many of us. The highly publicized plight of Sheri Finkbine of Arizona, the mother of four children all under the age of 7 years, who had unwittingly taken Thalidomide early in her fifth pregnancy and then was denied an abortion in this county and even in Japan, is easily recalled. Fortunately, she was able to obtain a legal abortion in Sweden, the fetus being described to her as "an abnormal growth that would never be a normal child."[9] Subsequently she had a normal fifth child. And who can forget the rubella epidemic of 1964-1965 and the seven reputable San Francisco Bay Area obstetrician-gynecologists who risked their reputations and their licenses to practice when they openly declared in a *Life* magazine article that they were interrupting pregnancies on fetal indications, a practice which at that time had no legal sanction? California led the country in recognizing the archaic nature of its old abortion law. The new law of 1967 gave legal protection to physicians who were performing so-called therapeutic abortions on fetal indication. Governor Ronald Reagan threatened to veto the bill unless specific reference to "fetal indications" was deleted. A compromise bill was passed which, in effect, allowed physicians some latitude without writing into law the concept that the embryo represents a full human person. This new law satisfied the medical profession, but no one at that time foresaw the impact of the women's rights movement and the subsequent 1973 landmark decision of the U.S. Supreme Court in *Roe v. Wade*. The court agreed, in essence, that up to the point of viability of the fetus, a woman had the right to control what was happening to her own body. The rights of the fetus were not equal until it was capable of maintaining an independent, extrauterine existence. Abortion on demand through the second trimester of pregnancy became the law of the land. In the 12 years since that time, over 16 million abortions have been performed in the United States. The number of abortions equals the number of live births in many areas of the country. But serious questions are still being asked about the morality of such a law.

The debate rages on—the pro-lifer advocates at one extreme and the pro-choice advocates at the other. The majority of the American public is somewhere in between, but the country has been polarized by this problem in a way that hasn't occurred since the slavery question almost destroyed the nation over 120 years ago. The emotional conviction the extremists bring to the abortion debate and the dogmatic positions they have taken have made a resolution of the problem impossible. Violence and terrorism have become the tractics of some. Individual physicians have entered or stayed away from the abortion arena according to their own consciences. One of the most vociferous and most visible has gone from one extreme to the other. A former director of the largest abortion clinic in the country resigned after having supervised over 60,000 abortions. In 1974 he wrote, "We must work together to create a moral climate rich enough to provide for aborition, but sensitive enough to life to accommodate a profound sense of loss."[10] He has now been completely converted and has aligned himself with the pro-life contingent, actively campaigning against abortion just as militantly as he did in 1969 when he helped organize the National Abortion Rights Action League. He says that he has seen the light, and an extremely biased videotape of an abortion pictured in real-time sonography, entitled "The Silent Scream," is his weapon. This "about face" brings to mind Arthur Koestler's comments[11] about the convert:

> To say that one had "seen the light" is a poor description of the mental rapture which only the convert knows (regardless of what faith he's been converted to). The new light seems to pour from all directions across the skull; the whole universe falls into patterns like the stray pieces of a jig-saw puzzle assembled by magic at one stroke. There is now an answer to every question, doubts and conflicts are a matter of the tortured past—a past already remote, when one had lived in dismal ignorance in the tasteless, colorless world of those who don't know. Nothing henceforth can disturb the convert's inner peace and serenity—except the occasional fear of losing faith again, losing thereby what alone makes life worth living, and falling back into the outer darkness, where there is wailing and gnashing of teeth.

The American public is deeply troubled by the abortion debate. A *Newsweek* magazine poll[12] taken this year revealed that 58% would support a ban on legalized abortion except in cases of rape, incest, and danger to the mother's life, but even more significantly, 40% have doubts about whether their position is right, regardless of whether they support or oppose it.

Kristin Luker,[13] associate professor of sociology at the University of California, San Diego, has published the

results of her own study of the abortion question—a study that she admits was conducted in order to explore her own feelings. *Abortion and the Politics of Motherhood* compares the socioeconomic structure and values of those individuals who are most active in the pro-life and the pro-choice movements. It reveals that women involved in each group, by and large, are from entirely different walks of life. The pro-choice advocates are career women who are trying to balance positions in the work force with reproduction. Those who consider homemaking and childrearing their primary goals make up a large part of those who actively condemn abortion. Each faction believes that if the other were to triumph, their own lives would have less meaning. Dr. Luker concludes that "it is not surprising that the abortion question has generated so much heat and so little light." Daniel Callahan,[14] director of the Hastings Center (Institute of Society, Ethics and the Life Sciences), says that the kindest thing one can say about the more monomaniacal supporters of either the pro-life or the pro-choice position is that they deserve each other.

One thing about abortion is certain. It is a fact of life—a reality. The morality of abortion cannot be legislated. It is, always has been, and always will be a tragic choice. As with so many judgments in medical practice, the decision involves a choice of the least bad alternative. Abortion can be morally justified provided it is done to relieve suffering and to ease the burden to the patient, her family, and to society. Our responsibility is to recognize the sovereignty of individual consciences—to help our patients consider all the ramifications of their predicament so that they can choose the course they can live with. If that choice involves termination of the pregnancy, then it should be done as early as possible, with safety, compassion, and a deep sense of loss for the life that is sacrificed.

Artificial insemination, surrogate mothering, and in vitro fertilization all have troublesome moral implications. There is little ethical concern with artificial insemination using the husband's semen (AIH). Artificial insemination with donor semen (AID) is another matter. Although it has been practiced for over 40 years, there remain questions regarding the legitimacy of the resulting offspring. A conservative estimate of 8000 babies each year result from impregnation by donated sperm. The conscientious physician involved in this type of activity has, in past, made an attempt to match the physical characteristics of the donor and the infertile husband, as well as to exercise due care and caution in selecting donors with the proper Rh type and in avoiding major genetic problems. The identity of the donor is protected, and some even mix the donor's and the husband's semen. Now, however, there are sperm banks offering frozen sperm for sale. Even do-it-yourself instructions are available for any women who desires to bear an infant—the so-called "baster babies," because in some instances insemination is carried out using a meat baster to introduce the semen into the vagina.

Of almost 400 practitioners of donor artificial insemination surveyed,[15] 80% use medical students or resident physicians almost exclusively as donors. One, in fact, had used a single donor for 50 pregnancies. This practice has caused some critics to claim that these physicians are making eugenic decisions and using what they consider to be "superior" genes for donor artificial insemination. They object that although the physician may believe that society needs more of the attributes possessed by physicians, it is unlikely that society does. Well, I say, better the attributes of doctors than those of lawyers. It doesn't seem to cross the minds of these critics that medical students and residents merely serve as a readily available and reliable source of donor semen. Moreover, in our increasingly egalitarian society, medical students represent a very broad cross section.

Some sperm banks fill over 100 orders a month, and some deal directly with the consumer. The Oakland, California, Feminist Women's Health Center has its own sperm bank and serves "all women regardless of their race, marital status, or sexual orientation." Single women, lesbians, and women with infertile partners are invited to participate. This organization estimates there are between 15,000 and 20,000 conceptions each year by donor artificial insemination and that 10% of these are in unmarried women or lesbians.

In Escondido, California, the Repository for Germinal Choice obtains and freezes sperm only from donors who meet rigid intellectual requirements. In 1982 it announced the birth of its first baby, the offspring of a woman with a high intelligence quotient and a Nobel prize–winning mathematician. Subsequently it was learned that the mother and her husband had served a prison sentence for fraud.

Aldous Huxley and George Orwell described a future world in which couples use donor artificial insemination voluntarily, using a central sperm bank supplied by carefully selected donors to increase the general intelligence quotient, or one in which donor artificial insemination is mandatory, with all marriages requiring advance approval by the "Party."

There is no question of the need for public discussion of the ethical and legal questions posed by donor artificial insemination, including its effect on marriage, on the family, and particularly on the welfare of the offspring. Should donor artificial insemination be carried out only by physicians, and if so, how can such a policy be enforced? Can one prohibit the artificial insemination of single women or lesbians on the basis that married couples make better parents? To do so

would be to discriminate because of marital status, a violation of a current legal principle. Obviously, some regulation of donor artificial insemination is necessary, and this should take the form of developing proper screening standards within the profession. Sperm banks should be held to the same standards.[16] The recent report[17] from Australia that it was "highly likely" that four women were exposed to human T cell lymphotropic virus type III (HTLV-III) from donor artificial insemination using semen from one particular donor makes this even more imperative. The California State Director of Public Health has ordered that all donors be tested for the acquired immune deficiency syndrome virus antibody. The similarity between the two acronyms, AID and AIDS, is positively eerie!

Surrogate motherhood is an outgrowth of artificial insemination. Several years ago a married woman in Dearborn, Michigan, infertile because of a hysterectomy, wanted a baby. An unmarried female friend volunteered to be impregnated by artificial insemination, using the husband's semen, and all three lived together during the subsequent pregnancy. This case was widely publicized, and the attorney who handled the case received many requests for a similar arrangement from other infertile couples. Surrogate motherhood-for-pay was the next step, and it wasn't long before a physician in Kentucky had about 100 women willing to be surrogate mothers. The fees ranged from $5000 to $10,000 each for the volunteer woman and for the attorney handling the legal arrangements. Moralists believe that surrogate motherhood represents an abuse of the lower socioeconomic class, from which most of the volunteers have been recruited. Some have looked on such contracts as baby selling. Most states have statutes prohibiting the placement of a child for adoption in return for a fee. The Michigan statute has been challenged because of the fear that it might prevent the payment of a surrogate mother. The petition has been denied, but the judgement is under appeal. The American College of Obstetricians and Gynecologists guidelines for physicians on surrogate motherhood state that there are "significant reservations about this approach to parenthood."

Nevertheless, surrogate motherhood is flourishing, and I have in my practice a young woman who is infertile because of a hysterectomy. She recently acquired an infant from a surrogate mother in Los Angeles and is very happy with the arrangement. Nevertheless, there have been some problems. In one recent case[18] a dispute arose when a microcephalic child was born and no one wanted to accept it. Eventually the surrogate mother and her husband accepted responsibility when it was learned that she had unwittingly conceived by her husband prior to being inseminated with the contracting man's semen. Suppose the surrogate mother changes her mind and decides to keep the baby. If she is married, her husband is the presumed father, and legally she could almost certainly keep the baby. If she is unmarried, there is a possibility that she could successfully sue the sperm donor for child support. This scenario has been outlined by an attorney ethicist[19] who says that the old adage "Mama's baby, papa's maybe" can be applied to the legal reaction to a surrogate mother who changes her mind. He has rather cleverly discussed the issues of contracting for a baby in terms of the Uniform Commercial Code as it might apply.[20] The code specifies that "if the goods or the tender of delivery fail in any respect to conform to the contract, the buyer may (a) reject the whole, (b) accept the whole, or (c) accept any commercial unit or units and reject the rest" (sec. 2-601). It also states that "if the seller gives no instructions within a reasonable time after notification of rejection the buyer may store the rejected goods for the seller's account or reship them to him or resell them for the seller's account" (sec. 2-604).

Louise Joy Brown, born in England on July 25, 1978, was the first known baby to result from in vitro fertilization, the result of efforts by Drs. Patrick Steptoe and Robert Edwards. Howard and Georgeanna Seegar Jones opened the first such clinic in the United States, in Norfolk, Virginia, and on December 28, 1981, Elizabeth Carr became the first baby born in this country after in vitro fertilization. There are now more than thirty such clinics in the United States, and more than 500 pregnancies have been recorded.

Paul Ramsey,[21] a theologian–medical ethicist, wrote in the *Journal of the American Medical Association* in 1972 that he considered in vitro fertilization to be "unethical medical experimentation on possible future human beings and therefore subject to absolute moral prohibition." The late Andre Hellegers,[22] of Georgetown University and director of the Kennedy Institute of Medical Ethics, thought that in vitro fertilization posed a threat to marriage and the family and, furthermore, that research in this area would lead to ethically undesirable research on late-stage fetuses.

Despite these concerns, in vitro fertilization is now firmly entrenched as a method of overcoming the infertility of women with bilateral tubal obstruction. Few now object to the use of this new technology for this purpose. Superovulation with harvesting, fertilization, and transfer of multiple ova is the technique that most clinics use to enhance the probability of a successful intrauterine pregnancy. However, a question has arisen as to whether or not it might be better to freeze the embryos for transfer in a subsequent cycle inasmuch as the drugs used for superovulation may interfere with implantation during the same cycle. It would also be more economical and would reduce the risk of multiple pregnancy. Serious ethical dilemmas have arisen from

the practice of freezing embryos for later transfer. The Rios case in Australia, in which a wealthy, infertile couple was killed in an airplane accident and left their frozen potential "heirs," illustrates the point. If only one zygote is transferred and implantation occurs, what does one do wth the fertilized eggs in the freezer? Should they be discarded? Is it appropriate to use them for experimental purposes?

The American Fertility Society guidelines state that it is ethical to experiment on human embryos fertilized in vitro—but only prior to the appearance of the primitive streak, the anlage of the neural tube, at about the fourteenth day. It allows the discarding of eggs up to that time and insists that strict rules define ahead of time the fate of frozen embryos. As of April of this year, eight babies—two in The Netherlands and six in Australia—have resulted from the transfer of thawed frozen embryos.[23] Several American clinics are trying cryopreservation techniques. One medical observer of in vitro fertilization in the United States[24] predicts that human embryos will eventually be grown in the laboratory beyond the blastocyst stage and that research activities will center on gene expression (including the role of oncogenes), chromosome segregation, cell and organ differentiation, implantation, and fetus-environment interaction. Because of the pressures to learn as much as possible about the process of human reproduction, even attempts at complete ectógenesis should not be ruled out.

The Joneses' clinic in Norfolk, Virginia, now has a waiting list of more than 8000 couples (a backlog of 16 years at the present rate) and plans to transfer about 2000 fertilized eggs this year.[25] The director of research at this clinic is the same individual who, at the National Institutes of Health, succeeded in producing normal, term pregnancies in castrated surrogate monkeys through embryo transfer and hormone replacement therapy.[26]

Let's look at what human reproductive technology has accomplished. With the advent of effective contraceptives, we had sex without babies, and with in vitro fertilization, we now have babies without sex. A possible combination of in vitro fertilization, artificial insemination with donor semen, and surrogate mothering would involve as many as five different "parents": a man and a woman to donate sperm and ova, a surrogate mother to receive the embryo, and an entirely different couple to adopt the baby. The question is, who is looking out for the interests of the baby?

One philospher[27] strikes an optimistic note, however. He believes that the traditional technique of "making babies" will remain the method of choice because "it's inexpensive, can be performed at home, takes little time, training, or skill, and is more fun."

What are the ethical problems associated with genetic counseling and prenatal diagnosis? First, there is the problem of the increase in the numbers of torts filed against physicians ostensibly for failing to inform at-risk patients of the availability of genetic screening tests. Second, there have also been claims as a result of falsely negative screening tests. Should a defective child be born, the physician may be held liable for the entire cost of raising the child. Moreover, the affected child may sue for "wrongful life." Until recently, none of the suits has been successful; however, the California Supreme Court ruled in 1982 that such a child could recover damages for medical expenses and special training. Obstetricians increasingly are finding themselves in a "catch 22" situation. The question has been raised as to whether or not a handicapped child can sue its parents for wrongful life if an abortion were refused after prenatal diagnosis of a severe malformation. How can one conclude that a handicapped life is worse than nonexistence? On the other hand, is there moral justification for intentionally bringing a severely handicapped child into the world?

Many in the medical profession operate on the assumption that the only purpose of prenatal diagnosis is to detect fetuses with major anomalies so that they can be aborted. The pro-life advocates, of course, object to prenatal diagnosis for this reason. They either ignore or are unaware of the fact that more than 95% of women who undergo prenatal testing are found to have unaffected babies and can be reassured. Thus the number of abortions is actually decreased by prenatal diagnosis. Without the knowledge and reassurance gained by negative test results, many women are not willing to take even a slightly increased risk of carrying an affected baby. Futhermore, prenatal diagnosis serves the purpose of detecting problems in the fetus that might be remedied or ameliorated during the prenatal period. Intrauterine fetal blood transfusion for erythroblastosis fetalis is, of course, the classic example, but more recently other problems, such as fetal urinary tract obstruction, have been tackled successfully. The era of intrauterine treatment of the fetus is just beginning. What about the couples who would not consider abortion no matter what anomaly might be discovered? In some clinics such an attitude might be considered grounds for refusing to carry out the tests because of the increased risk of inducing abortion. This risk should be balanced against the fact that the discovery of an affected baby would help to prepare the family, as well as the obstetrician and the pediatrician, for the birth of a compromised child.

Another ethical question raised by prenatal diagnosis is that of sex selection, that is, of intentionally aborting a fetus of an unwanted sex. Surveys have revealed that most couples who plan more than one child prefer the firstborn to be male. Firstborn children are better

achievers. A widespread practice of choosing a male baby as the first, and perhaps the only, child would reinforce sexist attitudes, to say nothing of the social consequences of an unbalanced population. On the other hand, with the existing imbalance favoring women in the comparative longevity of the sexes, such a practice might bring about a more equal distribution of men and women in the older age group. Despite these considerations, in our present society, sexism is wrong; therefore abortion for this reason is wrong.

In trying to come to some conclusion regarding medical ethics as applied to the practice of obstetrics and gynecology, I made an attempt to go back to the basics of moral philosphy and try to learn something about ethical theory. I did so after reading that some medical ethicists believe that without a serious inquiry into ethical theory, there is little point in describing or trying to resolve ethical dilemmas. The theoretical concepts encountered were those of utilitarianism prompted by John Stuart Mill, the categorical imperatives of Immanuel Kant, the doctrine of natural law as outlined by Thomas Aquinas and promoted by the Roman Catholic Church, and the determinism of Spinoza. Reading the treatises of Mill wasn't too bad, but if any of you have tried to read the translations of the original writings of Kant, you will know why I gave up in frustration and decided to accept the interpretations of contemporary philosphers. There are several excellent summaries of ethical theory now available. All these ethical theories have features that have been incorporated by various authors into a consideration of medical ethics, but each theory has shortcomings when it comes right down to a consideration of specific cases. There are purist philosphers who adhere to one theory to the exclusion of others, but even drawing on all theories poses difficulties in developing a set of universally applicable moral laws. One is left with the need for eclecticism in drawing up a series of ethical principles that one can use in daily practice. Then I remembered that one of my heroes, Albert Schweitzer, physician, theologian, philosopher, musician, and winner of the 1954 Nobel peace prize, struggled for many years to resolve philosophical questions of ethics. He said: "All that I had learned from philosophy about ethics left me in the lurch. The conceptions of the Good which it had offered were all so lifeless, so unelemental, so narrow, and so destitute of content that it was quite impossible to bring them into union with the affirmative attitude."[28] This from an individual who had spent several years of his life in the preparation of his doctoral thesis in philosphy on the works of Immanuel Kant. Well, if a physician of the caliber of Albert Schweitzer couldn't find a common thread in various ethical theories, all of which had been well developed prior to his time, how can we be expected to do it? In Schweitzer's case, he had a moment of inspiration that solved the dilemma for him. His description of that moment is illuminating. It was after Schweitzer had earned his medical degree and gone to French Equatorial Africa as a medical missionary. He wrote:

I was staying with my wife on the coast at Cape Lopez for the sake of her health—it was September 1915—when I was summoned to visit Madame Pelot, the ailing wife of a missionary, at N'Gomo, about 160 miles upstream. The only means of conveyance I could find was a small steamer, towing an overladen barge, which was on the point of starting. Except myself, there were only natives on board, but among them was Emil Ogouma, my friend from Lambaréné. Since I had been in too much of a hurry to provide myself with enough food for the journey, they let me share the contents of their cooking pot. Slowly we crept upstream, laboriously feeling—it was the dry season—for the channels between the sandbanks. Lost in thought I sat on the deck of the barge, struggling to find the elementary and universal conception of the ethical which I had not discovered in any philosphy. Sheet after sheet I covered with disconnected sentences, merely to keep myself concentrated on the problem. Late on the third day, at the very moment when, at sunset, we were making our way through a herd of hippopotamuses, there flashed through my mind, unforeseen and unsought, the phrase, "Reverence for Life." The iron door had yielded; the path in the thicket had become visible. Now I had found my way to the idea in which affirmation of the world and ethics are contained side by side.[28]

James Brabazon,[29] Schweitzer's biographer, points out the simplicity and naïveté and yet the great power of this concept—that there is a complete interdependence of all living things—a concept that is being increasingly recognized and accepted universally. As Brabazon[29] says:

Reverence for Life commands nothing. It forbids nothing. All it requires is that whatever is done should be done in full and deep awareness. Unlike creeds and codes, which provide human beings with preconstructed decisions and take away the need for choice, Reverence for Life lays on everyone the responsibility for every action.

Yet, in contemporary literature on medical ethics, little is said of Schweitzer's contribution. Perhaps it's because present-day philosphers think that his concept is too simplistic. Well, at least it was something that I could understand.

"Reverence for Life" is an idea that may be simple and naive, as some have claimed. On the other hand, it may be extremely profound. It obviously didn't mean to Schweitzer that all life should be preserved at any costs, for he recognized that where life is harmful to other life, a choice must be made. I am certain that he

would have abhorred the use of modern medical technology to artificially prolong human life at the expense of continued pain and suffering. Moreover, he was not an antivivisectionist, as "Dear Abby"[30] would have us believe, but strongly believed that laboratory animals should not be made to suffer or to be experimented on needlessly. He believed that pain-relieving medication was a necessary part of animal exploration involving pain or discomfort. If it were necessary to sacrifice a life, he thought that an awareness of the price paid should enhance the value of the life for which it was given. Schweitzer's philosophy, in just three words, eloquently expresses a doctrine that is particularly appropriate for those of us who are so intimately involved in the process of human reproduction. It may demand nothing or require nothing, but it does compel a deep and abiding respect, not only for the beginning of life, but for the middle and the end. It means that we must continue to seek after knowledge and sharpen our scientific skills. In our fascination with the science of medicine and rapidly advancing technology, we must never lose sight of the fact that we profess to care for patients. It means that we must communicate with the patient and her family, respecting the sovereignty of her desires, her beliefs, and her aims and goals. It means that we must weigh all decisions first according to patient benefit, for, in general, what's good for the individual patient is good for society. It means, also, that we must realize—and discuss openly with our patients—the uncertainties that make obstetrics and gynecology an art. Finally, it means that we must be wary of moral extremists who believe that there is one morality that fits every situation, acknowledging that only those whose medical decisions allow for individual differences are meeting the deeper demands of the ethics of our profession.[31]

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Estrogen use and cardiovascular disease

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In general, epidemiologic data from case-control and cohort studies have suggested that postmenopausal estrogen use confers a moderate degree of protection from coronary artery disease. The authors report reductions in all-cause mortality rates and in mortality rates for acute myocardial infarction among estrogen replacement users in comparison with never-users. It is calculated that even a small decline in the mortality rate for coronary artery disease among estrogen users would have a major impact on the overall risk/benefit ratio associated with estrogen use. (Am J Obstet Gynecol 1986;154:1181-6.)

Key words: Estrogens, postmenopausal, coronary artery disease

Cardiovascular disease is the number one cause of death in the United States, accounting for about 650,000 deaths annually (Surgeon General's Report,[1] 1979). The possible role of estrogens in modifying the occurrence of cardiovascular disease has long been controversial. Despite decades of debate, we remain so ignorant about the role of estrogens in the etiology of cardiovascular disease that recent reviews of the risks and benefits of estrogen replacement therapy have included this disease either as a hazard[2] or as a benefit[3] of such therapy, or the issue has simply been ignored.[4] There is experimental evidence that can be interpreted as supporting both viewpoints, but the available epidemiologic literature strongly supports a beneficial effect, although the magnitude of the associated protection is undetermined.

There have now been eight published case-control studies that have contributed meaningful information on the relationship between estrogen replacement therapy and cardiovascular disease, but only a few of these studies were designed specifically to evaluate this relationship[5-12] (Table I). Of the eight studies, seven have reported a reduced risk of cardiovascular disease in estrogen users, although in the earliest of these studies the risk approached unity after adjustment for other cardiovascular disease risk factors.[6] In the one other study[9] the small number of cases (n = 17), the young age of the patients (under 50 years), and their very high frequency of cigarette smoking (90%) complicated interpretation of the results.

Our case-control study of death from ischemic heart disease[5] was conducted as part of a series of case-control studies examining the risks and benefits of estrogen replacement therapy in a southern California retirement community. The 133 female residents of this community who died of cardiovascular disease over a 5-year period were compared with women of the same age who died during the same period but from causes thought to be unrelated to estrogen use and with women of the same age who were still living in the community at the time of death of the patients. Medical records were the source of information on estrogen use. With either control group used for comparison, estrogen use was associated with a substantial reduction in the risk of dying from ischemic heart disease. The relative risk with living control subjects used for comparison was 0.4 (95% confidence interval = 0.2 to 0.8); with deceased control subjects used for comparison, the relative risk was 0.6 (95% confidence interval = 0.3 to 1.0). Although information on other important cardiovascular disease risk factors was not consistently recorded, protection was conveyed to a comparable degree in women whose medical records indicated that they had other cardiovascular disease risk factors (including a history of cigarette smoking, obesity, stroke, hypertension, diabetes, angina, or a previous myocardial infarction), as in those women for whom there was no such evidence.

There have been five published cohort studies of estrogen replacement therapy and cardiovascular disease[13-17] (Table II). Two of these studies involved specific patient populations[14, 15] and showed reduced risks of ischemic heart disease in estrogen users. The three large population-based cohort studies[13, 16, 17] have given conflicting results. In the Framingham study[13] the number of observed deaths from cardiovascular disease

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Table I. Case-control studies of estrogen replacement therapy and coronary heart disease

Investigator, year	*Data source*	*Relative risk*	
		Ever use	*Current use*
Rosenberg et al.,[6] 1976	Interviews	NR	0.5
Pfeffer et al.,[7] 1978	Pharmacy records	0.9	0.6
Jick et al.,[9] 1978	Interviews	NR	4.2
Rosenberg et al.,[10] 1980*	Interviews	0.7	0.5
		1.5	0.9
Ross et al.,[5] 1981†	Medical records	0.4	NR
		0.6	NR
Bain et al.,[8] 1981	Mailed survey	0.9	0.7
Adam et al.,[11] 1981	Physicians	0.6	0.8
Szklo et al.,[12] 1984	Interviews	0.5	0.4

NR = Not reported.

*Rosenberg et al.[10] reported women aged 30 to 44 years (top) separately from women aged 45 to 59 years.

†Ross et al.[5] used two control groups for comparison: living controls (top) and deceased controls.

Table II. Cohort studies of estrogen replacement therapy and coronary heart disease

Investigator, year	*Description of cohort*	*End point*	*Relative risk*
Burch et al.,[15] 1974	737 hysterectomized women	CHD	0.4
Hammond et al.,[14] 1979	610 hypoestrogenic inpatients and outpatients	CHD	0.3
Gordon et al.,[13] 1978	2873 female residents of Massachusetts	Fatal CHD	1.0
		CHD	1.6
Petitti et al.,[16] 1979	1675 white women	MI	1.2
Bush et al.,[17] 1983	2269 white women	Fatal CHD	0.3

CHD = Coronary heart disease; MI = myocardial infarction.

in estrogen users was equal to the number of expected deaths from the same cause in the same group; however, the number of estrogen users was small. In the Walnut Creek Kaiser Permanente study, Petitti et al.[16] reported a relative risk of 1.2 for myocardial infarction among users of noncontraceptive estrogen; however, participants in this study were generally young, and only 26 cases of myocardial infarction were observed during follow-up, with nine of these cases having occurred in estrogen users.

Results from a well-designed cohort study were recently reported by Bush et al.[17] The association of estrogen use with all-cause mortality was examined in 2269 white women, aged 40 to 69 years, who had been followed up for an average of 5.6 years in the Lipid Research Clinics Program Follow-up Study. The relative risk of death was 0.4 in estrogen users in comparison with nonusers. The relative risk of fatal cardiovascular disease in estrogen users in comparison with nonusers was 0.3.

Because of the importance of clearly establishing the degree of protection from cardiovascular disease afforded by estrogen replacement therapy, we began, in 1981, a prospective study in the same retirement community in which our previous case-control research had been conducted. This study design provided the opportunity of obtaining details on potential cardiovascular disease risk modifiers, such as prior heart disease, hypertension, and cigarette smoking, as well as on dose and duration of estrogen replacement therapy.[18]

In June, 1981, a detailed health questionnaire was mailed to all permanent residents in Leisure World, Laguna Hills, a retirement community near Los Angeles, California. Residents of this community are almost entirely white, moderately affluent, and well educated; the residents' median age is 74 years, and about two thirds are women. After three mailings and follow-up telephone contact, 11,888 (62%) of the 19,209 residents returned questionnaires; 7610 of these respondents were women.

The cohort is being followed up for all hospital admissions to three hospitals serving the area (the vast majority of these admissions occur in one hospital immediately adjacent to the community) and for deaths recorded at the Orange County Health Department and the community business office. In addition, we have conducted a biannual remailing to the cohort; the first such mailing was conducted in 1983 and revealed a loss to follow-up of just 38 members of the cohort (0.4%).

As of August 31, 1984, 435 deaths had occurred among the 7610 women who returned our initial ques-

Table III. Age-adjusted mortality rates per 1000 and number of deaths (N) by any use of estrogen replacement therapy

	Never (N)	*Ever (N)*	*p Value*
All causes	20.5 (242)	17.1 (193)	0.06
Acute MI	4.8 (56)	2.6 (28)	0.007

MI = Myocardial infarction.

Table IV. Age-adjusted acute MI mortality rates per 1000 and number of deaths (N) by other cardiovascular risk factors and use of estrogen replacement therapy

	Estrogen replacement therapy	
Risk factors	*Never (N)*	*Ever (N)*
Previous MI/angina		
No	3.8 (38)	2.0 (19)*
Yes	10.7 (18)	5.2 (9)
Previous hypertension		
No	3.6 (24)	2.0 (14)*
Yes	6.4 (32)	3.0 (14)*
Smoking		
Never	4.9 (39)	2.0 (13)*
Ever	4.7 (17)	4.1 (15)

MI = Myocardial infarction.
*$p < 0.05$

tionnaire. The age-specific and age-adjusted death rates from all causes and from acute myocardial infarction are given in Table III. There were 242 deaths in never-users of estrogen and 193 deaths in ever-users, yielding a relative risk of 0.84. This reduction in the total mortality rate among ever-users was of borderline statistical significance ($p = 0.06$). There were 56 deaths from acute myocardial infarction among never-users, in comparison with 28 deaths among ever-users. The reduction in relative risk (0.54) among ever-users of estrogen was highly significant ($p = 0.007$). The majority of deaths in both never-users and ever-users occurred in the age decades of 70 to 79 years and 80 to 89 years. A substantial degree of protection from death caused by acute myocardial infarction was observed in both of these age groups.

The expected number of deaths from all causes and from acute myocardial infarction among ever-users was calculated with the use of the age-specific mortality rates among never-users. Among ever-users, there were 229 deaths expected, an excess of 36 deaths over the number observed. A total of 54 deaths from acute myocardial infarction were expected among ever-users, in comparison with 28 observed, an excess of 26 deaths. Thus a decreased number of deaths from acute myocardial infarction accounted for nearly three fourths of the overall reduction in the mortality rate among ever-users.

Among never-users of estrogen replacement therapy, a previous history of high blood pressure and an acute myocardial infarction or angina were both associated with a higher mortality rate from acute myocardial infarction, whereas there was no clear difference in the acute myocardial infarction mortality rate between persons who have ever smoked (the majority of whom were ex-smokers) and those who have never smoked, among different groupings by Quetelet's index or by hysterectomy status. In all these categories, ever-users of estrogen had lower acute myocardial infarction mortality rates (Table IV), and in most the observed reduction in the mortality rate was substantial.

The finding of a protective effect against death from acute myocardial infarction in estrogen users is consistent with the secular changes in the mortality rate for this disease. The cardiovascular disease mortality rate has declined nearly 30% in the past 20 years.[1] The cause of this decline has been attributed both to improved medical care and to changes in life-style.[19] It is probable that multiple factors are involved, since the decline has occurred to a comparable degree in both men and women and in both whites and nonwhites, and it is doubtful that either life-style changes or access to improved health care has occurred equally in all four groups during this period. Nonetheless, a marked decline in cardiovascular disease mortality in women is consistent with the increased prevalence of estrogen use since 1960. Since men have shown a similar decrease in mortality rates, other factors associated with the risk of cardiovascular disease must have declined to a greater degree in men than in women. One factor, the

Table V. Estimated changes in mortality induced by high- and moderate-dose estrogen replacement therapy, ages 50 to 75 years

Condition	*High dose*		*Moderate dose*	
	RR	*Cumulative change in mortality/100,000*	*RR*	*Cumulative change in mortality/100,000*
Osteoporotic fractures	0.4	−563	0.4	−563
Gallbladder disease	2.0	+3	1.5	+2
Endometrial cancer	7.0	+378*	2.0	+63*
Breast cancer	1.5	+938	1.1	+187
Ischemic heart disease	1.0	0	1.0	0
Net change		+756		−311
Net % change		+6%		−2%

RR = Relative risk.

*Case fatality rate for estrogen-induced endometrial cancer estimated at 0.05.

prevalence of cigarette smoking, did, in fact, decrease 26% in men but only 8% in women between the mid-1960s and mid-1970s.[1]

The finding of a reduced risk of cardiovascular disease among estrogen users is also consistent with other, but not all, related epidemiologic observations. For example, several studies[13, 20] have found an increased risk of cardiovascular disease in women undergoing an early artificial or natural menopause, strongly suggesting that ovarian function influences risk. On the other hand, an *increased* risk of vascular disease in women who use oral contraceptives is well documented. This high risk is observed over a wide range of vascular conditions, including venous thromboembolism, myocardial infarction, thrombotic stroke, hemorrhagic stroke, and subarachnoid hemorrhage.[21-24] On the basis of data from the Royal College of General Practitioners' large cohort study,[25] the risk of vascular disease, overall, was found to be increased about fourfold in oral contraceptive users in comparison with nonusers. The effect is especially strong in oral contraceptive users who smoke.[26, 27] However, recent data from this study and others[28, 29] demonstrate convincingly that the risk of arterial disease is related to the progestin rather than the estrogen component of the pill.

The biologic effects of estrogens are numerous, but the most likely mechanism to explain their protective effect on cardiovascular disease is their ability to alter lipid and lipoprotein levels. In the Lipid Research Clinics study of postmenopausal estrogen users,[30] the median serum triglyceride level was increased 26% and serum cholesterol was decreased 4%. Larger changes were observed in the lipoprotein cholesterol fractions than in the total cholesterol levels. The median high-density lipoprotein cholesterol level was 10% higher and the very low-density lipoprotein cholesterol level was 23% higher in estrogen users then in nonusers, whereas the median low-density lipoprotein cholesterol level was reduced by 11%. Other researchers have found even larger decreases in low-density lipoprotein cholesterol levels and even larger increases in high-density lipoprotein cholesterol levels in postmenopausal women treated with estrogen replacement.[31]

It is impossible to consider the role of estrogens on cardiovascular disease risk outside the context of other known or possible risks and benefits of estrogen replacement therapy. Since 1981, there have been at least 20 published studies on the relationship between estrogen replacement therapy and endometrial cancer. Nearly all these studies found a strong association between past estrogen use and development of this disease. Generally, it has been found that women using moderately high doses of estrogen for moderately long periods increase their risk of endometrial cancer by a factor comparable to that by which moderate cigarette smokers increase their risk of lung cancer.[32-34] Despite the experimental evidence and indirect epidemiologic evidence that estrogens are intimately involved in the pathogenesis of human breast cancer, the epidemiologic literature on breast cancer and estrogen replacement therapy is confusing. In the last few years there have been six population-based epidemiologic studies that have reported on this association. As a group, these studies find little or no increased risk of breast cancer with estrogen use overall, but they do suggest that long-term use carries with it perhaps a 50% increased risk of breast cancer.[35-40] On the other hand, there are now at least five case-control studies demonstrating that women who use estrogen replacement therapy substantially reduce their risk of sustaining postmenopausal fractures.[41-45] These studies suggest that fracture risk is lower with long-term estrogen use than with short-term use and that use for 5 or more years approximately halves the risk of nontraumatic osteoporotic fractures.

There are currently few available data on the effect of cyclical progestational agents given in conjunction with estrogen on each major component of the risk-

Table VI. Estimated changes in mortality induced by high- and moderate-dose estrogen replacement therapy, ages 50 to 75 years

Condition	*High dose*		*Moderate dose*	
	RR	*Cumulative change in mortality/100,000*	*RR*	*Cumulative change in mortality/100,000*
Osteoporotic fractures	0.4	−563	0.4	−563
Gallbladder disease	2.0	+3	1.5	+2
Endometrial cancer	7.0	+378*	2.0	+63*
Breast cancer	1.5	+938	1.1	+187
Ischemic heart disease	0.5	−5250	0.5	−5250
Net change		−4494		−5561
Net % change		−33%		−41%

RR = Relative risk.
*Case fatality rate for estrogen-induced endometrial cancer estimated at 0.05.

benefit equation. It is probable that progestational agents can minimize or even negate the carcinogenic effect of estrogens on the endometrium. It has been demonstrated that 12 or 13 days of progestational therapy each month can completely eliminate adenomatous hyperplasia,[46] a probable precursor of endometrial cancer. Although progestational agents appear to have their own beneficial effects on bone resorption,[47] there is no evidence that they further enhance the effectiveness of estrogen alone. On the other hand, progestational therapy may have a negative impact on the breast cancer and cardiovascular disease components of the equation. Mitotic activity in breast epithelium reaches a peak in the luteal phase of the menstrual cycle,[48] suggesting that progesterone, when it acts in conjunction with the luteal-phase estradiol peak, may be a breast carcinogen. As noted above, the risk of vascular disease in oral contraceptive users is clearly related to the progestational component of the pill.[28, 29] Progestational agents have the opposite effect of estrogens on high-density and low-density lipoprotein cholesterol.[49] Although hydroxyprogesterones may be less hazardous than other progestogens in relation to lipid secretion and metabolism, nonetheless they appear to totally negate the reduction in low-density lipoprotein cholesterol levels and the increase in high-density lipoprotein levels afforded by unopposed estrogen therapy.[49]

Despite the multiplicity of effects associated with estrogen therapy, if the risk of cardiovascular disease is influenced by postmenopausal estrogen use, as epidemiologic data suggest, in terms of mortality rates this would be the single most important factor in the cost/benefit equation, even if the relative change in risk is small.

One can crudely compute the effects on mortality rates, through the age of 75 years, of high-dose (1.25 mg conjugated estrogen) and moderate-dose (0.625 mg conjugated estrogen) estrogen replacement therapy given at 50 years of age to 100,000 representative U.S. women. Assuming the best available measures of incidence and 5-year survival rates for the relevant diseases and the following relative risks for high- and moderate-dose estrogen replacement therapy, respectively—fractures, 0.4, 0.4; gallbladder disease, 2.0, 1.5; endometrial cancer, 7.0, 2.0; breast cancer, 1.5, 1.1; and cardiovascular disease, 1.0, 1.0—one would expect high-dose therapy to produce a net *increase* in deaths of about 6% and moderate-dose therapy to produce a net *decrease* in deaths of about 2% (Table V).

If cardiovascular disease risk was reduced by 50%, the net decrease in the mortality rate would be in excess of 33% and 41% with high- and moderate-dose therapy, respectively (Table VI). These calculations stress the importance of determining with certainty any dose-response relationship between estrogen use and cardiovascular disease. Even in a weak dose-response relationship, the net mortality benefit to be derived from high-dose therapy would converge with that to be derived from low-dose therapy. A stronger dose-response relationship might favor high-dose therapy as a means of reducing mortality rates for estrogen-related diseases.

All these projections are crude and depend on a variety of assumptions. Nonetheless, these calculations suggest that even minor relative changes in the risk of cardiovascular disease following estrogen use can have a major impact on the overall risk/benefit equation. Just as it is important for clinicians to consider the possible effects of estrogen therapy on the entirety of the risk/benefit equation, it is also important that epidemiologists continue to refine these calculations through research aimed at measuring the effects of long-term low-dose versus high-dose therapy on the risk from cardiovascular disease, assessing the effects of combined estrogen-progestogen therapy on risk and clarifying the interactions between the effects of estrogen replacement therapy and the effects of known risk predictors of cardiovascular disease.

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Fetal injury prior to labor: Does it happen?

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Major advances have been made in the identification and prevention of perinatal factors that lead to long-term handicap or neurologic deficits. When the infant or child exhibits a major handicap, secrutiny of the pregnancy management often occurs in an attempt to define the causal factors. The medical goal of this inquiry is to prevent injuries and, when possible, to eliminate these factors. In the litiginous sense, any deviation from optimal, ideal care or any unusual observations, such as unusual or atypical fetal heart rate patterns, are often causally linked to the adverse outcome. There are at least four categories of major fetal injury that probably occur prior to labor. An awareness of, and a diligent search for, details will no doubt clarify the legitimate origins of many so-called birth injuries. Hence the common tendency to fixate on minor deviations and/or deficiencies of labor and delivery management as causing catastrophic injuries will be successfully challenged. (Am J Ostet Gynecol 1986;154:1187-93.)

Key words: Birth injury, fetal heart rate patterns, perinatal asphyxia

The problem of perinatal injury and compromised long-term outcome is understandably a topic of major concern and the focus of much current attention. Although many strides have been made in reducing the incidence of neonatal birth injuries, there is increasing concern over the rising use of cesarean section to achieve this benefit. Any long-term outcome that is less than perfect is likely to raise questions regarding the adequacy of pregnancy management, with particular emphasis focused on the labor and delivery process. Often, unrealistic public expectations, coupled with the current litiginous attitude, provoke critical scrutiny of compromised outcome that a few years ago would have been accepted as a result of the natural, but imperfect, reproductive process.

When a child suffers a major handicap or neurologic deficit, such as cerebral palsy, a process of inquiry should be initiated. There is a medical obligation to identify causal factors, with the goal being to minimize or eliminate such factors. Thus genetic assessment, metabolic evaluation, and scrutiny of antepartum events are often undertaken but are rarely definitive. Final focus then falls on the events of labor, delivery, and the newborn period. This detailed inquiry, unfortunately, often occurs in the form of medicolegal confrontation, rather than in an objective scientific investigation. The adversarial nature of this confrontation is charged with emotion and reflects biases that are oftentimes devoid of a truth-seeking, objective process.

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Currently the major contributor to adverse outcome is premature birth. Advances in neonatal care have dramatically increased survivorship of very low birth weight infants. However, such survivors exhibit a high incidence of morbidity, such as cerebral palsy, as evidenced by the fact that the incidence of cerebral palsy has not declined in recent years.[1] Thus any evaluation of compromised newborn-infant outcome must necessarily isolate infants with low birth weight from the appropriately grown term infants. Although similar disorders may occur in both groups, the process of obstetric intervention may be dramatically different for fetuses judged to be preterm or frankly immature. Furthermore, the expectations for the preterm infant and for infants exhibiting growth disorders and/or anomalies will be less than those expected for term-size infants.

The occurrence of fetal asphyxia and its ability to cause fetal death during labor has been well documented. Older reports in the literature attempted to define various time frames of pregnancy and their relationship to abnormal fetal outcome. Such studies necessarily antedated such technologic advances as fetal monitoring, ultrasonic diagnosis, and the newborn intensive care routinely available to such patients today. Moreover, such studies described group characteristics but could not provide individualized case assessment. Although peripartal asphyxia is an accepted cause of neurologic injury, the majority of such disorders are believed to have their origins apart from peripartal events.[2]

Labor management and, in particular, the evaluation

of fetal condition during the hours of labor are viewed with suspicion in the presence of known or documented neonatal compromise. Such evaluation is not unreasonable in view of the increased incidence of death and morbidity during this relatively short period. Within such a context, charted information is of particular importance, and the fetal heart rate (FHR) data obtained with the use of electronic fetal monitoring have become the primary commentary regarding fetal status. FHR patterns influence decisions affecting both the antepartum and the intrapartum management of pregnant patients. The primary benefit of FHR assessment is the correlation between patterns judged to be "normal" and subsequent favorable, or uncompromised, neonatal outcome. The observaton of what is judged to be other than normal lacks such strong prognostic value. Much of the criticism relating to FHR monitoring focuses on the lack of specificity of abnormal patterns, which, in turn, has often led to "unnecessary" intervention. On the other hand, when one is faced with an adverse outcome, particular attention if focused on FHR patterns, especially since they are thought to reflect fetal compromise. Patterns such as late deceleration, when present over the time-course of hours, are known to be associated with acidosis, depressed neonates, and neonatal morbidity. When such events have preceded a birth and are coupled with the observation of newborn encephalopathy—that is, seizures—it is reasonable to assume that such antecedent factors may have played a role in subsequent neurologic deficits.[3, 4]

Under other circumstances, the FHR tracing does not demonstrate a pattern recognized as being associated with abnormal outcome. The most notable examples of this type occur in the dying fetus or the fetus subsequently found to exhibit major congenital anomalies incompatible with life.[5] In a number of circumstances we have encountered nonspecific FHR findings that fail to portend severe fetal acidosis or severe compromise encountered in the neonate who was anticipated to be born "normal."[6]

The question then arises: Can "abnormal" FHR patterns observed during labor result from or indicate an injury that preceded labor? The documentation of such a fetal injury depends on the identification of a lesion that chronologically must have preceded labor. The majority of such lesions have been diagnosed at the time of postmortem examination of antepartum stillborn infants and thus preclude intrapartum observation. Neonatal deaths and their respective postmortem findings are often confounded by complications that these neonates encountered in the nursery. Fortunately the diagnostic advances of computerized tomography and of ultrasonic scanning in neonates during the first hours of life have provided new insights into antenatal injuries. Lesions can be diagnosed and dated as having occurred before labor, thus exonerating the labor process as the probable causal factor when an adverse outcome is confronted.[7] Critical evaluation of intrapartum events, including FHR, are of major importance in such cases.

The recent medical literature offers evidence that there are four major categories of fetal injury that may be documented as having occurred prior to the onset of labor or delivery[8-12]: (1) intracranial hemorrhage, (2) myocardial infarction, (3) sepsis and congenital pneumonia, and (4) meconium aspiration.

Unfortunately, little information exists relevant to the intrapartum FHR findings that are unequivocally associated with fetal injuries documentable as having occurred prior to labor. Herein, we will provide examples of intrapartum FHR tracings associated with injuries that probably antedated the labor process in term infants with appropriate growth for gestational age.

Fetal injuries

Intracranial hemorrhage. Demonstration that an intracranial hemorrhage occurred during fetal life prior to labor can be made by two specific means. First, demonstration of gliosis in the brain of stillborn infants documents the chronicity of intracranial injury and hemorrhage and its necessary occurrence prior to labor.[8] Second, the demonstration of liquefication or a porencephalic cyst in the fetus or in the infant during the early newborn period provides documentation. This pathologic residual of infarction, or intracranial hemorrhage, requires a minimum of 7 days to evolve. The importance of early identification of this lesion is self-evident and may lead to a careful evaluation of the FHR characteristics associated with this chronic lesion. A recent report[11] has described the initial "normal," followed by abnormal, FHR findings associated with a major maternal complication and apparent fetal decerebration. The subsequent death and autopsy findings in this infant confirmed the timing when the insult occurred. Immediately after decerebration, the fetus demonstrated persistent lack of variability, and the intrapartum tracing showed minimal FHR variability and an absence of late decelerations.[11]

Myocardial infarction. The occurrence of myocardial infarction in the perinatal period is well known.[12] Postmortem studies during a recent 42-month period at our institution have demonstrated the occurrence of perinatal myocardial infarction in 35 cases (Turkel, S., personal communication, 1985). Findings consistent with infarction were found in 18 cases ending in five stillbirths and the deaths of 13 neonates within less than 24 hours of age. The minimal time required for the development of typical histologic findings of myocardial infarction is in excess of 24 hours. Hence these injuries

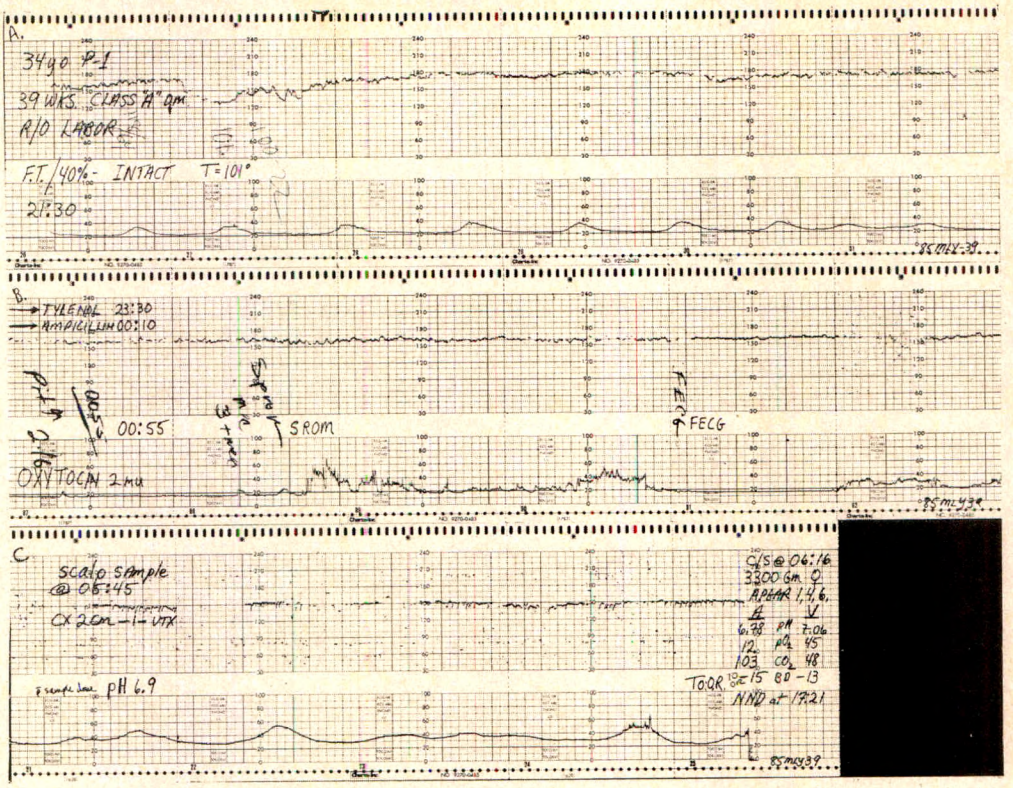


Fig. 1. Intrapartum FHR uterine activity findings associated with a fetus having intrauterine sepsis. Chronologic times may be noted in the left-hand portion of each panel.

probably occurred prior to the labor process. Five such infants weighed over 3000 gm, and five were judged small for gestational age. Unfortunately, we have been unable to identify (or retrieve) pathognomonic FHR tracings in term-size infants suffering infarctions.

Congenital infection. Sepsis in the term neonate is usually successfully treated with minimal long-term compromise. The occurrence of an early neonatal death after which a necrotizing pneumonitis was demonstrated at autopsy implies a fetal disease process of several days' duration.[10] The following case example illustrates such a case and the associated findings.

The patient was a 34-year-old woman, gravida 2, para 1, admitted at 39 weeks in early labor and with a temperature of 101° F. Physical examination demonstrated intact membranes, positive fetal movement by observation, and fingertip cervical dilation with 40% effacement, with a vertex presentation at a −3 station. The remainder of the physical examination was noncontributory, other than the abdominal examination, in which fetal weight was estimated at 3600 gm. Urinalysis and laboratory tests failed to identify the source of fever. Portions of the intrapartum FHR tracing of this patient are presented in Fig. 1. An antipyretic agent was administered, as noted in Fig. 1, *B*, to reduce the maternal fever. Spontaneous rupture of the membranes occurred approximately 2½ hours following admission and revealed thick meconium, with gram-negative bacteria demonstrated by microscopic examination. The diagnosis of amnionitis was then made and ampicillin and gentamicin therapy initiated.

Six hours after the woman's admission and after 5 hours of oxytocin administration, the cervix had become completely effaced, 2 cm dilated, with the vertex at a −1 station. Because of persistent fetal tachycardia and decreased variability, a fetal scalp sample was obtained and revealed marked fetal acidosis (Fig. 1, *C*). A cesarean section was expeditiously performed and resulted in the delivery of a depressed 3300 gm female infant. The Apgar scores were 1, 4, and 6 at 1, 5, and 10 minutes, respectively. The cord blood gas results are presented in the lower right portion of Fig. 1, *C*. The FHR findings revealed a baseline rate in excess of 160 bpm and failed to demonstrate definitive deceleration patterns throughout the course of labor. There was minimal beat-to-beat variability, as demonstrated in Fig. 1, *B*, and accelerations were not documented at any time during the 8½ hours of observed tracing. Newborn evaluation revealed a limp, cyanotic infant who had meconium suctioned from below the vocal cords twice. Resuscitative efforts were difficult. The baby could never be properly ventilated and died at 11 hours of age. Autopsy findings demonstrated necrotizing pneumonia, renal necrosis, and necrosis in additional

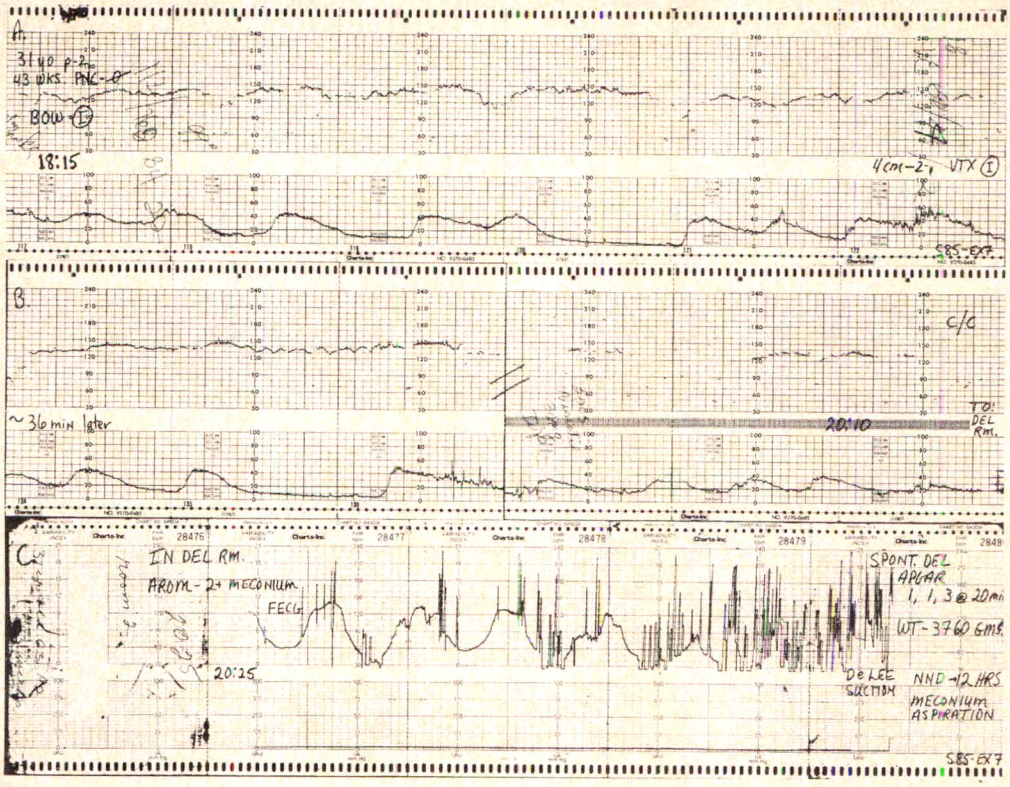


Fig. 2. The FHR uterine activity findings in a fetus that had experienced in utero aspiration of meconium. The time frame of occurrence may be noted in each panel, and the terminal direct fetal heart rate is presented in the bottom panel *(C)*.

organs. *Listeria monocytogenes* was cultured from both newborn and autopsy specimens. The mother's postoperative course was uncomplicated.

Meconium aspiration. The occurrence of meconium aspiration is a major cause of morbidity in the otherwise "normal" term-size neonate. This complication occurs more commonly in postdates pregnancies. The occurrence of meconium aspiration in utero preceding the onset of labor has been previously documented.[13] Histologic observation of macrophages containing meconium particles, in the lung, implies 24 hours or more tissue exposure to meconium. Likewise, the association of a baseline FHR is excess of 150 bpm, minimal variability, and unanticipated severe fetal acidosis and neonatal morbidity has been reported. In such cases the FHR patterns often lack episodes of acceleration or dramatic deceleration.[6] The following case illustrates the findings associated with probable meconium aspiration in utero.

The patient was a 31-year-old woman, gravida 3, para 2, who presented at 43 weeks' gestation having received no prenatal care. Physical examination performed on admission revealed a temperature of 99° F, with cervical examination showing 30% effacement, 4 cm dilation, and intact membranes with the vertex at a −2 station. FHR tracing was obtained with the use of external methods in Fig. 2, *A* and *B*. Total time of observed labor was <2 hours, as noted at the end of the Fig. 2, *B*, when the cervix was completely dilated and the patient was taken to the delivery room. When artificial rupture of the membranes revealed meconium, a fetal electrode was applied and demonstrated a markedly pathologic FHR pattern. Ten minutes later, as seen in Fig. 2, *C*, the patient was delivered of a 3760 gm infant with Apgar scores of 1, 1, and 3 at 1, 5, and 20 minutes, respectively. The fetus had undergone DeLee suctioning while on the perineum and was promptly intubated and vigorously resuscitated. In the neonatal intensive care unit a chest x-ray film revealed bilateral infiltrates consistent with meconium aspiration. The infant remained intubated and was mechanically ventilated but died at 12 hours of age. Postmortem examination of the lungs revealed extensive meconium aspiration, which was confirmed by histologic examination. In addition, acute funisitis was diagnosed, and numerous macrophages were noted in the sections from lung tissues. Perinatal asphyxia and extensive meconium aspiration were listed as the cause of death.

Comment

Currently there is unequivocal evidence that fetal injury can occur prior to the labor process. We present

intrapartum FHR tracings from selected examples of appropriately grown term neonates who apparently suffered an antepartum fetal injury. The intrapartum FHR findings often shared in common are a lack of FHR accelerations, an elevated baseline rate, decreased variability, and an atypical response or pattern lacking definitive decelerations. When the clinician encounters such findings in early labor, suspicion regarding fetal well-being should be raised.

Although these FHR findings cannot be looked on as pathognomonic of a fetal injury prior to the onset of labor and delivery, they should raise one's level of suspicion. As exemplified in our first case presentation, the occurrence of maternal fever confounds these particular observations. In several studies, intrapartum FHR tachycardia has been significantly associated with an increased risk of neonatal respiratory distress syndrome, asphyxia, and meconium aspiration.[14, 15]

The postterm infant who demonstrates the triad findings of an elevated baseline FHR, decreased variability, and meconium-stained amniotic fluid presents a unique problem. Historically, these fetuses have been noted to have increased morbidity and, often, to have FHR patterns that do not fit expected findings associated with fetal distress. Herein, we have presented a case with unusual and atypical FHR findings complicated by meconium aspiration and subsequent neonatal death. Meconium aspiration was documented at postmortem examination, and microscopic evidence suggested that aspiration had indeed occurred prior to the labor process.

In summary, we suggest that the observation of FHR patterns described in this report at the onset of labor should raise the clinician's level of suspicion that the fetus may have sustained a prelabor injury. This is particularly true if one has previously observed normal FHR or ultrasonic findings in an otherwise uncomplicated pregnancy. Thus the transition from normal to abnormal suggests that an adverse event has occurred in the fetus.

It is hoped that these observations and concepts will lead to a heightened awareness on the part of the clinicians in the search for FHR patterns that may indicate fetal injury prior to the labor process. To this end, careful antepartum, as well as intrapartum, observations are mandatory. Likewise, definitive neonatal evaluations, including creatine phosphokinase isoenzyme measurements, ultrasonic examination of the fetal head and/or heart, and other diagnostic procedures, must be promptly performed. Finally, should death occur, definitive evaluation for possible lesions and/or histologic evidence that documents the occurrence of an insult prior to labor, coupled with a detailed record of the labor process, including the FHR tracing, will no doubt clarify the probable time of occurrence in many abnormal outcomes. It is thus probable that the automatic assignment of blame to the labor and delivery process will be successfully challanged.

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Discussion

Dr. Peter Roberts, Portland, Oregon. Dr. Paul and his associates have written an interesting and informative paper. Until recently, almost all occurrences of brain damage, meconium aspiration, and neonatal sepsis have been attributed to intrapartum events. Thus, if there has been any serious central nervous system or pulmonary damage in a term newborn with appropriate growth for gestational age, its occurrence has been laid to the time of labor. The burden of proof for any injury has therefore been transferred to the obstetrician, and he must defend his labor management.

This article (along with those of other authors, notably Niswander and Manning) proposes, in some instances, the occurrence of fetal damage prior to the onset of labor. This may give some medicolegal relief. However, as pointed out by Dr. Paul, our real challenge is in continuing our search for the cause of these injuries so as to minimize and/or eliminate them. Until this is accomplished, we cannot be happy (and rest assured that the attorneys won't be, either).

In reviewing the well-documented cases of prelabor pulmonary sepsis and meconium aspiration, Dr. Paul has pointed out a clear and present danger signal—namely, the triad of thick meconium, elevated baseline FHR, and the lack of beat-to-beat variability. When this triad occurs in a term or postterm fetus, immediate delivery is mandatory, so that treatment can begin and further damage can be avoided.

Before asking Dr. Paul two questions, I wish to make a comment as a practicing obstetrician. Until accurate methods are available to precisely diagnose prelabor insults, every effort must be made to prevent any damage at the time of labor and delivery. By doing so, we will most importantly prevent further morbidity of the newborn—and at the same time, if any injury is present, there will be no doubt that the injury did not happen during labor.

I have two questions. First, current teaching has been that in the case of term infants with appropriate growth for gestational age, meconium aspiration occurs as the result of a vigorous respiratory effort and that efforts of that magnitude do not occur prior to labor and/or delivery. What mechanism do you believe causes aspiration before labor? Second, in the case of antepartum brain damage, do you foresee a procedure and or a protocol for the prelabor diagnosis of fetal neurologic damage?

Dr. Theodore Loring, Eureka, California. The presentation by Dr. Paul is timely and of great importance to our specialty at the present time. We are all aware of the problem of neurologic deficits in the newborn and its impact on malpractice premiums for obstetricians.

During the past 3 years I have served on a Nationwide Neurological Deficit in the Newborn Committee with representatives from the larger physician-owned malpractice insurance companies. This study is sponsored by the Physicians Insurance Association of America. To date we have studied about 450 cases of "brain-damaged infants" and have found the problem to be astronomical, representing about $2 billion dollars in reserves. Last May, at a Physicians Insurance Association of America meeting, Dr. Sanders, a pathologist at Michigan State University, presented a glimmer of hope in this problem. His studies of placentas of newborn infants born with neurologic defects revealed a condition he calls endovasculitis of the placenta. He believes that this is a long-standing placental change that possibly accounts for the neurologic damage. Since his articles have all been in the pathology journals, most obstetricians are probably not aware of endovasculitis of the placenta. I wonder, Dr. Paul, whether you have any information on, or opinion of, Dr. Sanders' work?

I urge all of you who are delivering babies to save the placentas of all babies with a low Apgar score, as well as the monitor strips. Someday that saved placenta might save you in court.

Dr. Robert Goodlin, Denver, Colorado. I recently moved to the University Hospital in Denver, and one of the things I was looking forward to was that babies there do not die from meconium aspiration. However, within a few months, there was a case that was managed ideally, for as soon as thick meconium was noted, and with a very low scalp pH value, the patient had an immediate cesarean section. There was no meconium below the cord, and the infant had been aspirated with the DeLee apparatus. Unfortunately, the infant died of meconium aspiration, which apparently was the first such case in 10 years. I just want to point out that even the well-known system, as used in Denver, is not without the rare failure, especially if the meconium aspiration occurs before the onset of labor.

There's an interesting paper in the *Australian and New Zealand Journal of Obstetrics and Gynecology*, entitled "The Battered Fetus." The author reports on a series of women from western Samoa who were delivered of fetuses with intracranial hemorrhage, and there was no history of trauma or anything else to explain the lesion. Whenever we're attempting to defend the occurrence of these cases, remember that the battered fetuses are fairly well established in the literature.

Dr. Thomas Moyers, San Diego, California. Speaking as a practicing obstetrician and one actively involved in caring for high-risk pregnancies, I believe that this study represents an especially important direction for current and future investigation. Knowledge of the timing of the insult that results in later evidence of fetal damage, or poor outcome of pregnancy, is an essential element in defending liability claims. More important, such knowledge may lead to changes in the management of pregnancy during both the prenatal and the intrapartum period, thus improving outcome. The availability of this kind of information in the obstetric literature is important for both reasons.

Dr. Julian Parer, San Francisco, California. I believe that this work is extremely important, in agreement with the other commentators. For some time now, we've been told that most cerebral palsy is not due to intrapartum asphyxia but, rather, to antepartum neonatal or infant events. My question is, Do you have some quantification of how much of this injury occurs before labor? That is, what percentage of damage evident at birth is due to relatively remote antepartum incidents?

Dr. Paul (Closing). I'd like to thank the discussants, particularly Dr. Roberts, and try to answer the questions. The first question was, What is the mechanism responsible for meconium aspiration prior to labor? I can't definitively tell you what causes this, but it is probably some type of fetal distress factor. It is speculation

to say that the baby had a cord accident or some other event, such as a maternal disorder, that stressed the baby. Whatever the cause, the baby probably undergoes a series of gasping efforts that result in the meconium's being aspirated into the lungs.

The second question, I think, regarded the development of a standard protocol to investigate such cases. We have no specific protocol, but when there is a high level of suspicion, we evaluate suspect cases. The suspect high-risk infants are the babies who are dysmature or inappropriately grown, and they're apparently most vulnerable to problems. Dr. Greggory DeVore is working in the area of fetal cardiac function. We see a repetitive pattern of cardiac dysfunction in the fetus when growth is impaired. The right side of the heart apparently begins to become dysfunctional, and then the left side is similarly affected. In the process, a number of such patients develop oligohydramnios. These fetuses are vulnerable to the stress of labor and are probably the ones who incur myocardial infarction prior to the actual birth. Measuring isoenzymes in the cord blood of such babies then becomes very important. This is a group we're very interested in exploring. Most babies with right-sided dilation in fetal life adapt promptly, as soon as they're born. At birth the left side begins to assist the circulation. The babies may appear normal in the nursery, and yet they may have had an injury. No one knows what the long-term outcome of such findings are.

Dr. Loring asked about placental endovasculitis and Dr. Sanders' work. I have no comment in reply. I'd only make the comment that the role of infection is something that we've underestimated. The two cases I've presented, both with intact membranes on entry to the hospital, had evidence of infection at necropsy. One patient had *Listeria* infection, and the other had funisitis without systemic sepsis. There is an apparent infectious process that I think we have not paid enough attention to in the past.

Dr. Goodlin commented about a recent case of in utero meconium aspiration in Denver. I'm glad they finally have had the case.

The early reports from Denver about the prevention of such problems prompted Frank Manning to describe in utero aspiration from our service. With our volume, we see the exceptions, and we saw such a case promptly. I guess their low delivery volume finally produced such a case.

I appreciated Dr. Moyers' comment. Dr. Parer, I can't tell how many injuries occur prior to, as opposed to during, labor. We're just trying to document case-by-case observations. I would say that the majority of injuries we're talking about occur prior to labor and delivery. They're often developmental or products of some dysfunctional growth problem. You must obviously separate out babies that are growth retarded or preterm from the term infants in such estimations. I'd say that most of these injuries probably occur prior to labor.

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Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus

Charles B. Chambers, M.D., and Joseph S. Unis, M.D.

Pomona, California

The sonographically enlarged uterine cavity in the postmenopausal woman has been described as indicative of uterine malignancy. In this study, in 10 of 15 cases of proven uterine malignancy with prior pelvic sonographic studies there was evidence predicting the diagnosis. In 10 of 12 cases with positive sonographic findings, uterine malignancy was found at operation. This reveals a 67% rate of success as to the value of negative sonographic findings and an 83% rate of success with positive findings. Pelvic sonography is not a screening test for uterine malignancy, but if positive findings are present, surgical investigation is indicated. (Am J Obstet Gynecol 1986;154:1194-9.)

Key words: Sonography, uterine malignancy, menopause

Since the beginning of gynecologic ultrasonography, various reports have appeared describing the sonographic appearance of the uterus and endometrial cavity in the normal uterus[1] and in the pathologic nonpregnant uterus.[2] The reported findings in the postmenopausal uterus indicative of endometrial adenocarcinoma have been the fluid-filled, obstructed uterus,[3, 4] the enlarged uterine cavity,[5] the enlarged uterus,[5, 6] and the lobular uterus with a mixed echo pattern.[6] The latter finding is reported as being found in Stage III and Stage IV disease. This paper reports on the sonographic findings in 15 patients with proven adenocarcinoma of the endometrium or uterine sarcoma who had prior pelvic sonograms. In 10 of these cases (66.7%) there were findings suggesting malignancy, whereas in the remaining five cases the reports were noncontributory. Also included in the report is a prospective study of 12 cases (the 10 mentioned above plus two other cases) in which there were sonographic indications of malignancy. The purpose of this study is

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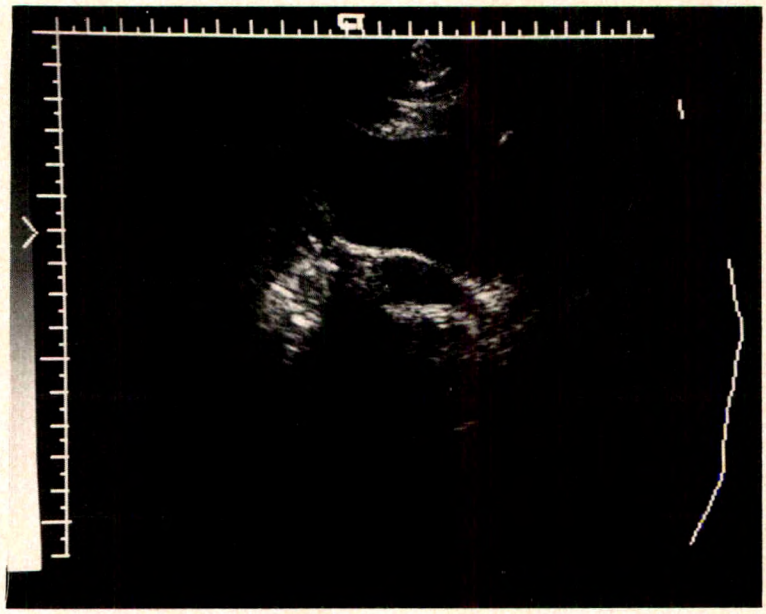


Fig. 1. Longitudinal sonogram of a normal postmenopausal uterus with uterine cavity not visible.

Table I. Preoperative sonographic findings in 15 patients with endometrial adenocarcinoma or uterine sarcoma

Patient No.	*Age (yr)*	*Sonographic findings*	*Findings at curettage or hysterectomy*
1	63	Markedly prominent endometrial echoes; uterus not enlarged	Well-differentiated endometrial adenocarcinoma with minimal myometrial invasion
2	63	Prominent endometrial echoes; uterus slightly enlarged	Well differentiated endometrial adenocarcinoma with less than one-third myometrial invasion
3	69	Enlarged uterus with diminished sound transmission or increased acoustic impedance; uterus appears homogenously involved	Curettage only; uterus 6 × 10 cm sounded to 6 cm; moderate amount necrotic tissue; poorly differentiated adenocarcinoma endometrium
4	61	Uterus diffusely enlarged with dense central endometrial echoes	Adenocarcinoma endometrium well differentiated with one-third myometrial invasion
5	80	Uterus enlarged; variable echogenicity in region of central canal	Adenocarcinoma endometrium, tumor mass measures 4.5 cm; extension to one-third myometrium
6	61	Large cavity with prominent echoes	Polypoid mass 9 × 8.5 × 5.5 cm; mixed mesodermal tumor omental metastases
7	53	Bulbous enlargement of endocervical area and proximal uterine body with increased echogenicity	Adenocarcinoma endometrium involving the lower portion of the uterine cavity and entire endocervical region
8	65	Marked increase in echogenicity of the central endometrial echo, which is also wider	Endometrial polyp 3 × 2.5 cm; well-differentiated adenocarcinoma endometrium with no myometrial invasion
9	66	Irregularly enlarged uterus with diffuse variable echogenicity	Rhabdomyosarcoma
10	75	Cavity too large for patient's age	Adenocarcinoma endometrium with cervical extension
11	53	Right adnexal mass 6 × 8 cm; left adnexal mass 2 cm; no mention of uterine size or cavity	Mucinous cystadenocarcinoma right ovary; serous cystadenoma left ovary; small superficial focus of adenocarcinoma endometrium
12	69	Uterus normal size and configuration	Multiple site papillary adenocarcinoma endometrium with one-third myometrial invasion
13	63	Uterus normal size, shape, and position	Adenocarcinoma endometrium Stage III; 4.5 cm cavity; large amount necrotic tissue
14	75	Uterus moderately enlarged and demonstrates calcification compatible with fibroids; cavity not mentioned	Adenocarcinoma endometrium filling cavity to wall of uterus with perforation; benign leiomyomata
15	55	Normal uterus with small cervical fibroid; uterine cavity not apparent	2 cm cervical leiomyoma; no cavity enlargement; plaquelike well-differentiated superficial adenocarcinoma endometrium

to evaluate this procedure by examination of these prospective and retrospective series.

Material, methods and results

Between January, 1980, and July 1, 1985, 101 patients with adenocarcinoma of the endometrium or uterine sarcoma were treated at Pomona Valley Community Hospital. Of these, 15 (14.4%) had pretreatment sonograms (Table I). During the same period the radiology department reported on 12 cases with sonographic findings suspicious for uterine malignancy (Table II). Sonographic scans were obtained on commercially available gray-scale units with 2.25 or 3.5 MHz transducers.

Comment

The postmenopausal uterus has been described as having an upper-limits anterior-posterior diameter of

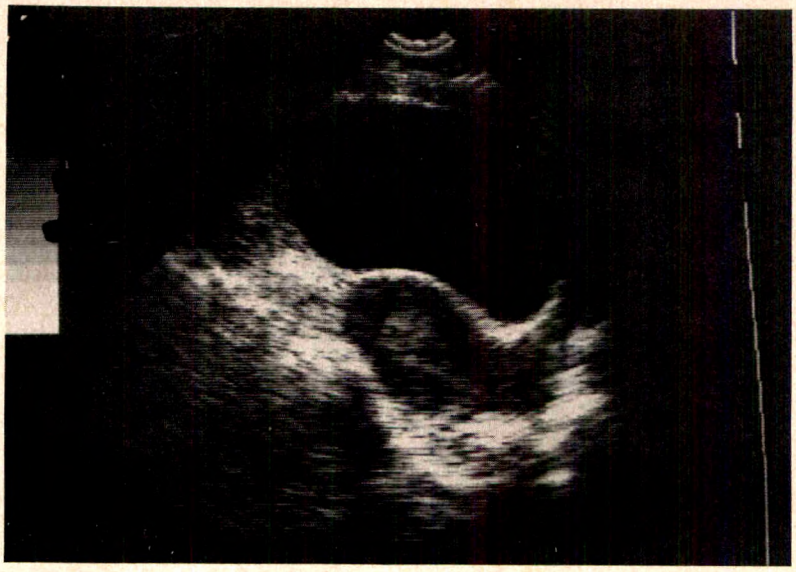


Fig. 2. Longitudinal sonogram of a postmenopausal uterus with variable echogenicity of the uterine cavity.

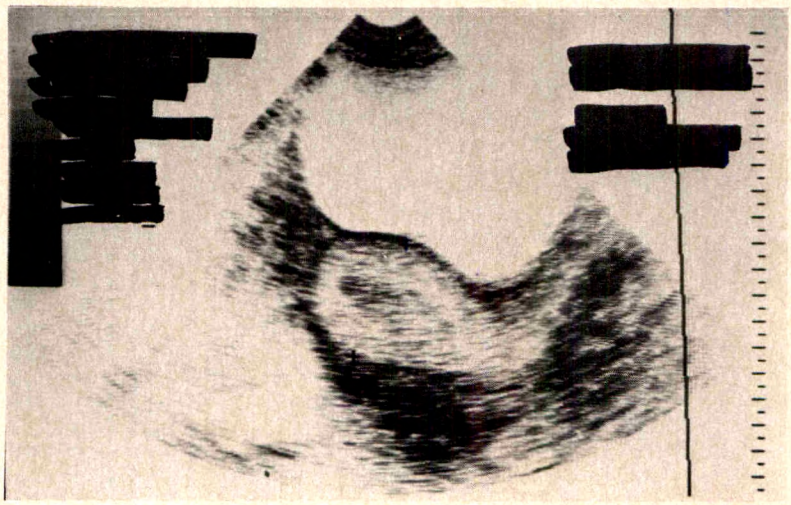


Fig. 3. Longitudinal sonogram of a postmenopausal uterus with an enlarged uterine cavity.

Table II. Pathologic findings in 12 patients with sonographic indications of malignancy

Patient No.	*Age*	*Sonographic findings*	*Findings at curettage or hysterectomy*
1-10		See Table I	See Table I
16	90	Prominent endometrial echo compatible with uterine malignancy	Surgeon unable to perform curettage because of stenosis; no malignancy at hysterectomy; atrophic endometrium; cavity not mentioned by pathologist
17	70	Slight increase in anterior-posterior diameter of uterus; prominent endometrial echo	Benign proliferative endometrium; endometrial polyp

3 cm and a length of 8 cm. There is a homogenous echo pattern (hypoechoic), and the uterine cavity is not visible (Fig. 1). In this series the sonographic changes indicative of uterine malignancy in the 10 positive cases were a prominent or variable echogenicity of the uterine cavity (eight cases) (Fig. 2), enlarged uterine cavity (four cases) (Figs. 3 and 4), enlarged uterus (four cases), and mixed echo pattern of the uterine body and lobular

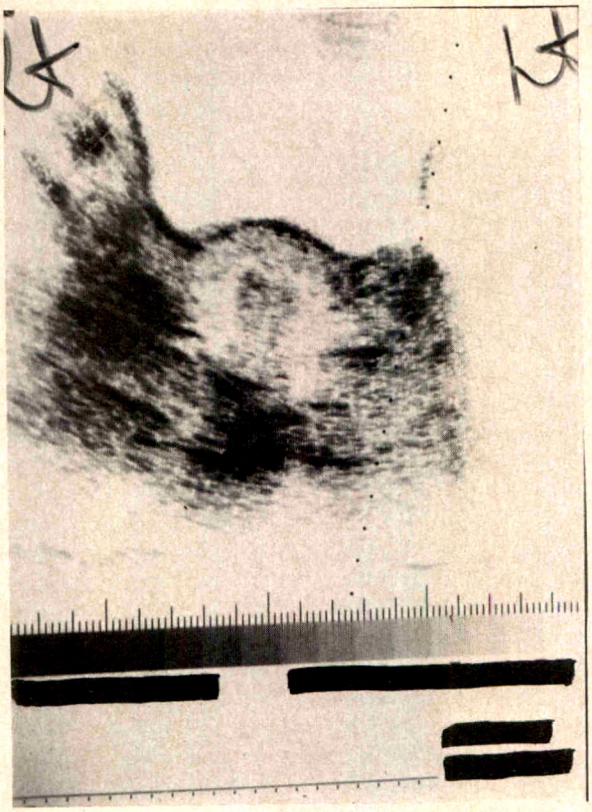


Fig. 4. Transverse sonogram of the uterus in Fig. 3.

uterus (two cases) (Fig. 5). The cases of mixed echo pattern and lobular uterus confirmed the report[6] that these changes were evidence of Stage III or IV disease.

Analysis of the five false-negative cases reveals two cases (No. 11 and No. 15) of a superficial endometrial adenocarcinoma with no cavity distortion, consequently there was no sonographic change. The other three cases are nonexplainable, since two cases (No. 13 and No. 14) had Stage III disease and case No. 12 had ⅓ myometrial invasion. With regard to the two false-positive cases, case No. 16 may be explained by cervical stenosis with probable distension of the uterine cavity and case No. 17 is explained by a benign endometrial polyp.

The primary conclusion from these series is that the pelvic sonogram does not serve as a screening test for endometrial adenocarcinoma, since 33% of the cases were missed. As a corollary, however, if a pelvic sonogram is performed and any of the suspicious findings as described are present, surgical investigation is definitely indicated. One question to be resolved is what changes there will be in the uterine cavity in the postmenopausal patient on cyclic estrogen-progesterone. This problem is being investigated by us at this time.

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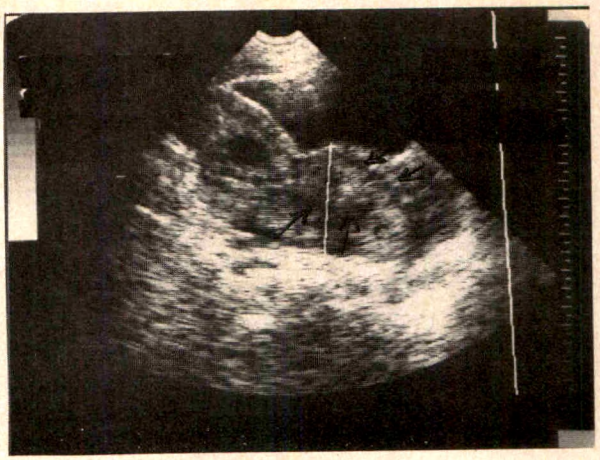


Fig. 5. Longitudinal sonogram of a lobular uterus with a mixed echo pattern of the uterine body.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Louis Bartolucci, San Francisco, California. Dr. Chambers has looked into the utility of gynecological ultrasonography in the diagnosis of endometrial carcinoma and uterine sarcoma, a very interesting subject that somehow failed to receive a fair share of attention in the recent, explosive development of this discipline.

It would be useful to know whether or not the sonographer who reviewed the scans used in the study was told of the diagnosis of endometrial carcinoma in advance or was otherwise oriented to the fact that the test was a screening procedure for this lesion. This knowledge would lend support to either way of looking at the reported cases, the retrospective or the prospective one.

Dr. Chambers draws some important conclusions from his study: The first is that ultrasound is not an adequate screening test for endometrial carcinoma because it missed the diagnosis in 33% of his cases. Aside from the general consideration that a diagnostic test capable of 67% efficiency should still be usable if one recognizes its failure rate, I would like point out some other facts that may mitigate this conclusion.

In 90% of the cases of the study in which ultrasound correctly diagnosed an endometrial malignancy, the uterine cavity was visualized and reported abnormal to a variable degree. In the two cases labeled as false positive, a distended cavity was seen in one (No. 16), probably secondary to cervical stenosis, and an endometrial polyp was found in the other (No. 17)—two conditions worth investigating in a postmenopausal uterus. It is more important, however, to observe that in all five cases in which ultrasound failed to diagnose an existing endometrial malignancy, the uterine cavity was not mentioned by the sonographer and presumably not visualized by the scan.

Ultrasonic visualization of the uterine cavity is a relatively easy procedure in a nonpregnant uterus if adequate scanning techniques are observed and modern standard instruments are used. Many authors have reported such techniques in detail,[1, 2] and recently developed, commercial ultrasonic scanners have resolving capability sufficient to show endometrial thickness and texture at various stages of the menstrual cycle[3] or fine motions of the endometrial lining during ovulation induction or other stimulations.[4] The postmenopausal uterus is no exception, except for cases of extreme atrophy or concomitance of large pelvic masses.

In my opinion, a sonographic study of a nonpregnant uterus should always include a description of its cavity, especially when the scan is aimed at detecting endometrial pathologic conditions.

I agree entirely with the second and third conclusions in Dr. Chambers' study. I wish to congratulate him on his valid effort to sharpen our thoughts on this application of gynecological ultrasonography and look forward to hearing about his observations on the postmenopausal endometrium under estrogen-progesterone replacement therapy.

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Dr. Charles Plows, Anaheim, California. I think one thing this paper points out is that patients do not have to be bleeding to have endometrial carcinoma. I recall one of my cases in which the patient had been treated for over a year for watery discharge but did not respond. She had an ultrasound scan of her pelvis which revealed a filling defect in the uterine cavity. On curettage it was found she did have adenocarcinoma.

The only thing I would ask Dr. Chambers is how many of these patients were symptomatic? I can understand that inability to assess adequately the pelvis would be indication for pelvic ultrasound studies.

This interesting paper brings to mind that a patient does not have to experience postmenopausal bleeding or irregular bleeding patterns to have adenocarcinoma.

Dr. Simon Henderson, San Francisco, California. I would like to ask Dr. Chambers whether he is actually proposing ultrasonography as a screening test for endometrial carcinoma; if he is, is he just applying this to patients with symptoms, and if not, does he think we should do this on everybody?

I would also like to comment on the use of the smaller diameter hysteroscopes that we now have available for making a diagnosis of endometrial carcinoma. The Hamou hysteroscope can be used in the office even in postmenopausal women with very small cervices. Paracervical block can be used and sometimes no anesthesia at all. Hysteroscopy has certainly been done in France in clinics for some time and is being used now in offices and clinics in this country for intrauterine diagnosis.

Dr. Conley G. Lacey, San Francisco, California. I, too, have been interested in the use of sonography for the diagnosis of pelvic masses and endometrial cancer. In reviewing this technique for the diagnosis of pelvic pathologic conditions several year ago at Tulane Medical Center, we found that sonography was often inaccurate and produced as many false positive findings as false negatives. In Dr. Chambers' series a false positive result was followed by surgery that caused the death of one patient. In my experience the misinterpretation of sonographic findings is a relatively common error in clinical practice, and at the two extremes of error, results in life-threatening delay in surgery on the one hand or unindicated laparotomy on the other.

From my experience in several institutions in the United States I have been exposed to many sonography departments. I have learned from this experience that the results of sonography are, of course, directly proportional to the skill of the sonographer. I have also found that most sonographers never see, or examine, the patient. They attempt to correlate what is found on pelvic examination done by someone else with what they see on the sonogram. Consequently, it is very important to correlate the clinical findings with the sonographic findings and not order the sonogram in the first place if there are other clinical imperatives that would suggest that the results of the sonogram will not alter what you do.

Dr. S. Gainer Pillsbury, Long Beach , California. There are situations where additional nonoperative evaluation of the endometrial cavity would be useful in cases of questionable findings on ultrasound. We have just installed a magnetic resonance imaging unit at our hospital and have taken some pictures of the uterus. You get absolutely beautiful, clear pictures of the endometrial cavity and the uterus in many instances. This might be of some value in cases where the findings are in doubt.

Dr. Joseph Yon, Seattle, Washington. I want to echo

what Dr. Lacey said about the poor results that we get a lot of the time, trying to evaluate pelvic masses with ultrasound, but I have had four patients in the past few years who, either because of their body habitus or vaginal stenosis, were difficult to examine, and none of whom had any vaginal bleeding. And I got the ultrasound scan just to see what it was I was not feeling. Two of them had Stage IV endometrial carcinoma and two of them had Stage I, and were diagnosed at curettage. And I would also echo what was said about nuclear magnetic resonance imaging. They are excellent; you can even tell the depth of myometrial invasion on some of the good NMR scanners, which is really going to be of help in preoperative evaluation of endometrial carcinomas.

Dr. Chambers (Closing). Dr. Bartolucci, Dr. Unis, who was the co-author with me on this paper, a radiologist, and I reviewed all the sonograms. But these reports were the reports made by the radiologist before the surgical procedure. In those five cases in which there were false negatives, we could not see any cavity on review of the cases, although this was not mentioned in the report.

Dr. Henderson, I am not proposing that gynecologic ultrasonography be a screening test. I do not feel that it is indicated. When one of my patients has postmenopausal bleeding, I am not going to order a pelvic sonogram if I can do an adequate pelvic examination and if I can do an endometrial biopsy, or if I have to do a curettage. But I find that with many of the family physicians and many of the internists, this is a means they turn to.

So we are going to have these sonograms and the point that I am making is that if we do get a report that gives a positive finding, then we should investigate it. I definitely agree with Drs. Pillsbury and Yon that nuclear magnetic resonance imaging is going to be an excellent test, but obviously, expense comes into play with it.

Partial colpocleisis

Charles F. Langmade, M.D., and Joseph A. Oliver, Jr., M.D.
Pasadena, California

Partial colpocleisis, in comparison with complete colpocleisis, offers several advantages. The most obvious advantage is the avoidance of recurrent prolapse in sexually nonactive, older patients. Historically, colpocleisis has been reported to produce stress incontinence, which may be avoided by partial colpocleisis. Careful attention to avoiding anterior wall adhesions by leaving anterior and posterior vaginal walls separated and a very small, nonfunctional vagina will prevent scarring with fixation at the bladder neck, which in most instances will result in stress incontinence. To leave a functional vagina in an elderly widow, not active for many years and with no plans for sexual activity, will leave the patient with the possibility of recurrent prolapse. Partial colpocleisis, a relatively short procedure with minimal blood loss and with careful attention to the avoidance of scarring between the anterior and posterior walls, will obviate the reported stress incontinence following complete colpocleisis. One hundred two cases from 1970 will be analyzed and reported. (Am J Obstet Gynecol 1986;154:1200-5.)

Key words: Prolapse, partial, colpocleisis

Berkely and Bonney[1] once stated that the possibility of curing a case of procidentia in which the entire uterus had been removed, without narrowing of the vagina and therefore preventing sex relations, is almost nonexistent. To correct this problem was of great importance to many gynecologic surgeons of the day. Le Fort,[2] in 1825, reported his operation, but this procedure was prone to produce postoperative stress incontinence and was done for uterine prolapse. Telinde, in discussing Phaneuf's 1952 article,[3] pointed out that in Le Fort's procedure and the modification by Goodall and Powers,[4] the scarring produced a downward pull at the bladder neck and contributed to stress incontinence. Symmonds et al.[5] and Phaneuf[6] did not address the problem of postoperative stress incontinence. Anderson and Deasy[7] presented 16 cases in which the patients underwent hysterocolpectomy by their method; of these patients, three had postoperative stress incontinence (personal communication). Goldman et al.[8] reported on 118 patients who underwent the Neugebauer-Le Fort operation[2, 9] and had a 10% incidence of urinary incontinence and three failures. Pratt and Baker[10] reported urinary stress incontinence following Le Fort's operation, as did Hanson and Keetle[11] and Heany.[12]

An operation designed to prevent any chance of recurrence and to prevent stress incontinence would be the ideal procedure. Patients can make the decision as to further and future sexual activities. Widows who are 80 years of age and who have been both widowed and nonactive for 20 years can certainly be fairly certain that they will not again become sexually active.

Material and methods

During the years 1970 to 1984, a total of 102 patients underwent a type of partial colpocleisis designed to prevent stress incontinence and also to ensure that there would be no recurrent prolapse. These patients ranged in age from 53 to 95 years, with an average age of 75.4 years. Fifty-one had had previous surgery, 30 abdominal and 21 vaginal surgeries. Six had had previous Cooper's ligament support of vaginal vault prolapse.[13] Twenty patients had preoperative urgency incontinence, and 23 had preoperative stress incontinence. Twenty-eight patients were taking imipramine (Tofranil), 25 to 75 mg, for urgency incontinence prior to surgery. Twenty-four patients had had pessary trials that were not satisfactory. All the patients used vaginal estrogen cream prior to surgery and did pubococcygeus muscle exercises. They were all taking oral estrogens unless breast problems contraindicated this therapy.

Operative technique. The two main procedures done were (1) vaginal hysterectomy, anterior and posterior repair, and partial colpocleisis in 60 cases and (2) anterior and posterior repair, enterocele repair, and partial colpocleisis in 42 cases.

In the vaginal hysterectomy group, the uterus was removed, the peritoneal portion of the enterocele sac was ligated high, and the uterosacral ligaments in the area of the ligaments were plicated in the midline with interrupted chromic 0 sutures. The area of the ligations is mentioned because if sections are taken through these areas many times, the ligaments will no

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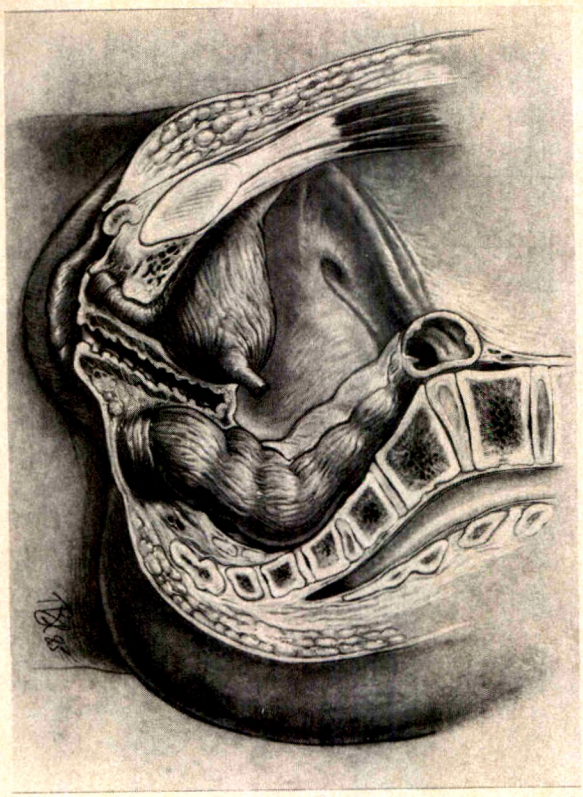


Fig. 1. A "pencil-sized vagina" is formed with adequate distance between the anterior and posterior walls to obviate scarring and downward pull at the bladder neck.

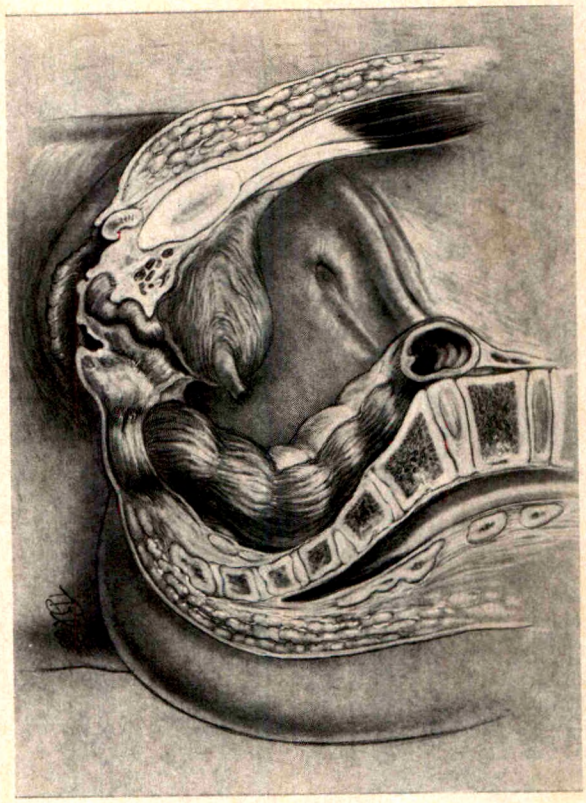


Fig. 2. Complete colpectomy leads to scarring, with distortion of the urethra and bladder neck and subsequent downward pull at the bladder neck.

longer exist. The anterior wall mucosa is dissected over the cystocele, and the vesicovaginal fascia is imbricated with chromic 0 interrupted sutures. The most important step in the anterior repair is to remove very little vaginal mucosa. Removing too much vaginal mucosa leads to scarring under the bladder neck, with subsequent downward pull and stress incontinence, as described by Phaneuf.[3] If scarring from previous surgery is present, it should be lysed and the mobility of the bladder neck established, especially in patients with stress incontinence. In the enterocele and cystocele group that had had previous hysterectomy, the treatment was the same.

The posterior repair is done in such a way as to form a pencil-sized vagina with the anterior and posterior vaginal walls remaining apart, thus excluding scarring in this area and postoperative stress incontinence (Fig. 1). If a complete colpectomy is performed, the lack of vaginal mucosa under the bladder neck leads to scarring and distortion of the urethra and bladder neck (Fig. 2).

A V-shaped incision is made high on the vulva between the labia minora and the labia majora (Fig. 3) and continued downward to the rectum. The uppermost part of the incision starts just below the urethral orifice and extends down to the midportion of the perineum, just above the rectum. A midline incision is made up to the peritoneal reflexion, the levator muscles are identified on either side, and the excess vaginal mucosa is removed. On the posterior wall, a considerable amount of mucosa is removed. The center area,

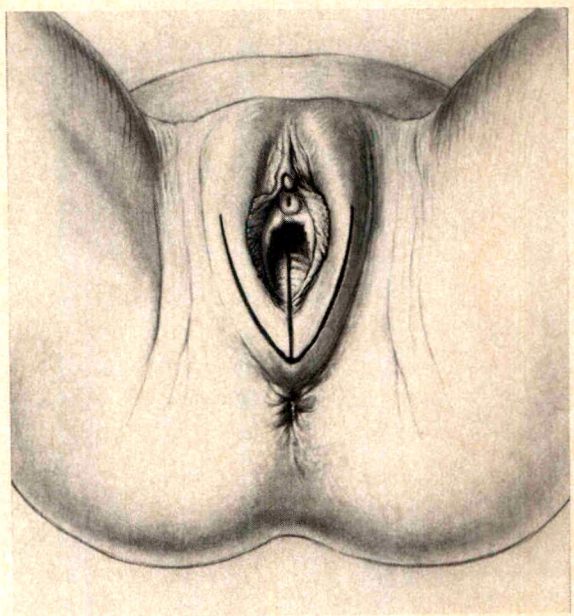


Fig. 3. A V-shaped incision is made high on the vulva between the labia minora and the labia majora down to the rectum.

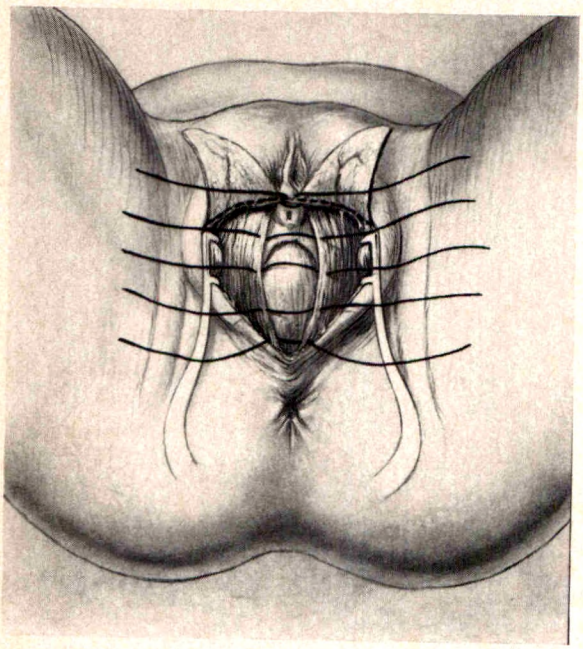


Fig. 4. The levator muscles are sutured in the midline from the urethra to the rectum.

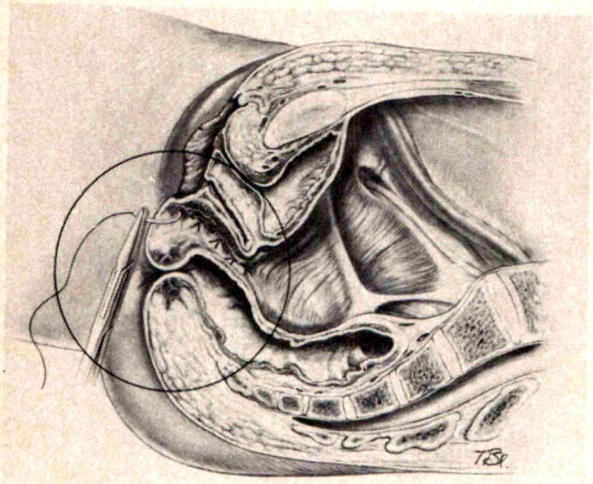


Fig. 5. The interrupted sutures are brought out on a straight line to the fourchette to greatly decrease the diameter of the vagina.

between the urethra and the rectum, is sutured with interrupted chromic 0 sutures on large Mayo needles (Fig. 4). The suturing is done from the peritoneal reflexion on a straight line out to the fourchette, greatly reducing the diameter of the vagina but leaving the anterior and posterior walls separated (Fig. 5).

The posterior vaginal wall is closed with continuous locked chromic 0, and more interrupted sutures close the superficial perineal muscles and fascia. The perineum is closed with subcuticular suture of the same

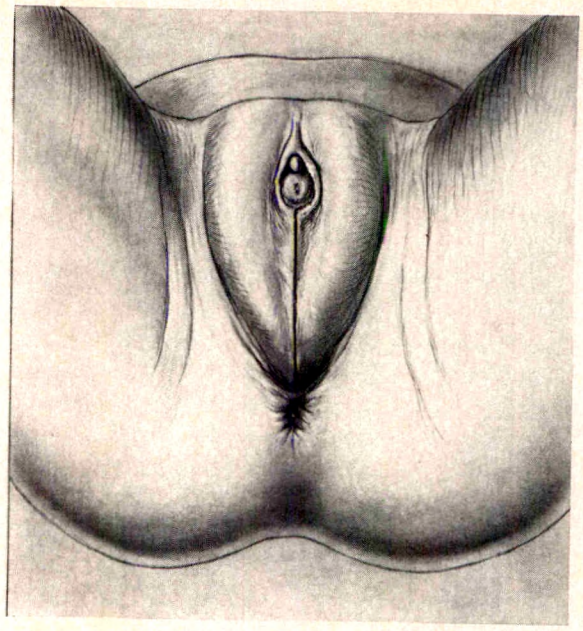


Fig. 6. The perineum is closed with a subcutaneous and subcuticular running suture up to the urethra. The opening just below the urethra admits the size of the little finger for about 10 cm.

chromic 0 suture used to close the posterior vaginal wall (Fig. 6).

Penrose drains were placed in the perineum in 74 cases.

Antibiotics were used postoperatively in all cases. Procaine penicillin, 1.2 million units, given intramuscularly every 8 hours, and streptomycin, 750 mg every 12 hours in six doses, were used prior to 1981. No severe complications from the streptomycin were noted, and only rare skin rashes from the penicillin were noted. In 1981, tobramycin sulfate was given, 80 mg as an average, every 12 hours intravenously, with aqueous penicillin, 2.5 million units, given every 6 hours intravenously. Patients who were sensitive to penicillin received clindamycin (Cleocin), 300 mg, given every 6 hours intravenously for 72 hours. One case of temporary diminished urinary output was attributed to tobramycin sulfate but resolved spontaneously in 4 days after the drug was discontinued.

Results

Failure. There were no failures or recurrent prolapse in any of the 102 cases. The follow-up varied from 1 year to 14 years, with an average of 2 years 11 months.

Operative time and blood loss. The operation is easy to perform and was done in an average time of 78 minutes. Patients were an average of 75.4 years old. It is well to have a short operating time and low amount of blood loss in this age group. The average blood loss

was 231 ml, which is a reasonable amount for this age group and a procedure accompanied by no failures.

Stress incontinence and urgency incontinence. Of the 102 patients, only two had stress incontinence postoperatively, and both of these patients had had stress incontinence preoperatively; in no instance was stress incontinence produced by the operation. Of the 20 patients with urgency incontinence, 15 had the same symptoms postoperatively.

Voiding time was from 3 to 21 days, with an average of 6.1 days. Those patients going home with a catheter were 16 in number, and of these, 10 voided on return to the office in 1 week with catheter removal, five voided in 2 weeks, and one in 3 weeks. All had an indwelling urethral Foley catheter No. 14 with leg bags. All eventually voided.

Hospital stay averaged 9.4 days, with a range of 6 to 19 days. The only cause of prolonged hospital stay was an elevated temperature.

There were no deaths in the series.

Complications. Two patients continued to complain of stress incontinence postoperatively. Both had had stress incontinence before surgery. Forty-three patients had urinary incontinence postoperatively; however 27 recovered and 15 continued to have urgency incontinence. One patient had urgency incontinence postoperatively who did not have it preoperatively. One patient had a temporary renal toxic reaction to tobramycin, which resolved spontaneously. One patient had pyelonephritis with septicemia, which cleared with treatment, but the patient was hospitalized for 19 days. This patient increased the average hospital stay a significant time.

No transfusions were given, and no hematomas or abscesses were diagnosed.

No patients changed their mind in regard to partial colpocleisis or sexual intercourse after surgery.

There were no cases of pulmonary embolism, and the one patient with a temperature of 104° F was diagnosed as having a drug reaction caused by sulfisoxazole (Gantrisin). All the patients discharged with catheters in were receiving some type of catheter coverage, such as Gantrisin or nitrofurantoin (Furadantin) and, in some cases, tetracyclines.

Forty-four patients had a postoperative febrile course, with an average temperature of 100.6° F in the first 72 hours, with subsequent reduction to normal within 24 hours. The range of temperature was from 100° to a high of 104° F. Six patients had an average temperature of 100.7° after 72 hours, with a range of 100.4° to 101.7° F. All patients were afebrile on discharge. Eleven patients had postoperative pyuria, but all patients went home with negative urine, even though 16 patients went home with an indwelling catheter.

Comment

One hundred two patients undergoing partial colpocleisis had no recurrent prolapse; there were only two cases of postoperative stress incontinence. There were no major complications in the series, and operating time was short and blood loss minimal.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Frank LeCocq, Yakima, Washington. In 1952 Emil Novak stated that he had not seen a case of vaginal prolapse that could not be corrected from below, but many voices have been raised since that time to dispute his assertion. Gynecologic surgeons have described the suspending of the vagina from the rectus fascia, the pubis, the sacrum, and Cooper's ligaments, as recommended by Dr. Langmade. All these procedures have been successful. A properly performed vaginal procedure again emerges as a primary repair for vaginal prolapse, and it takes advantage of the long-recognized fact that perineal tissues heal rapidly and with little reaction.

This discussion allows me to say a word about today's somewhat tenuous state of vaginal surgery. Dr. Dave Nichols, of Providence, Rhode Island, recently noted the absence of vaginal surgeries in reviewing American Board Candidates' case lists. Vaginal surgery may indeed be the victim of the increasing amount of scientific material crammed into the residency years. Of increas-

ing importance with the vaginal approach is the usual reduction in days of hospitalization. In this era of diagnosis related groups, millions of dollars are saved with each day's reduction.

This Society can boast of having leaders in the surgical repair of vaginal prolapse, since over the years the contributions of William Bradbury and Jess Rust, as well as of Charles Langmade, have been presented to this Society.

Drs. Langmade and Oliver have presented an impressive number of patients who must be divided into two distinct groups. The first sixty patients described are suffering from uterine procidentia and have been treated with the conventional vaginal hysterectomy, with excision and ligation of the enterocele sac, and with a partial colpocleisis. The second forty-two cases were diagnosed as vaginal prolapse, and the patients were possible candidates for the author's previously described Cooper's ligament suspension. In previous papers discussing Cooper's ligament suspension, the author has alluded to the treatment by vaginal colpocleisis, but the numbers and results have not been recorded. Thus the author has not ignored the vaginal approach in the past but now evaluates its advantages.

I wish to make a few remarks about technique. In 1961 the late William Bradbury, in his usual charming manner, presented a paper on subtotal vaginectomy for the treatment of vaginal prolapse. He noted that, in ancient times, Euryphone suspended the patient by her feet for 24 hours as an appropriate treatment for vaginal prolapse. The success of this treatment is unreported, but I suspect that no further complaints were forthcoming from the patient. Scarring of the tissues with a red-hot iron or needles was also an ancient treatment for vaginal prolapse.

In 1887, Léon Le Fort, speaking to the Paris Surgical Society, presented the first successful surgical procedure for vaginal prolapse. The disadvantage of covering the cervix, producing a nonfunctional vagina and, all too frequently, stress incontinence, has largely removed the procedure from our armamentarium. A modified Le Fort operation, called a lateral Le Fort by Cusier, may allow a functional vagina. One of our members, Morton Stenchever, of the University of Washington, is an advocate of this approach and reports excellent results with no recurrences. He stresses, however, limiting the proximation of the anterior and posterior walls at the bladder neck, thus avoiding urinary incontinence, and he further stresses "self-esteem" when the patient can observe that she still has a vagina.

For many years we have utilized a technique similar to that described by Dr. Langmade with considerable success. Care must be taken to complete a high ligation of the entire enterocele sac to avoid recurrence of prolapse or, rarely, a so-called anterior enterocele. We have also observed that the presence of a vagina, although minuscule, might result in a better self-image for the patient. If stress incontinence is severe, the Pereyra-Stamey-Raz procedure can assist with little additional operating time. Originally proposed by Randall and Nichols in 1981, suturing of the vaginal apex to the right sacrospinous ligament is helpful in the vaginal approach. This procedure has been successfully performed and described by Warren Jones and Joe Markee, of our Society.

We differ in the choice of suture materials, using the polyglycolic acid materials instead of catgut. Drains are not used in the perineum as described, but only at the vaginal apex in an extraperitoneal position. I question whether drains are necessary in the perineum, in view of the excellent and usual healing of perineal tissues.

Routine prophylactic antibiotics are not used except in cases of premenopausal vaginal surgery or if postoperative morbidity occurs. Have the authors found the routine use of antibiotics to be of enough value to offset the drug complications that they describe herein? Needless to say, the indwelling catheter always warrants prophylactic urinary antibiotics. The suprapubic cystocatheter improves the postoperative bladder management and does not require medication, but it does require more monitoring.

In regard to sexual function, I always remember our beloved babysitter, who had an orange-sized procidentia, the kind that makes the surgeon's eyes gleam and operating room nurses gasp. After she assured me of having had many years of celibacy, a vaginal procedure similar to Dr. Langmade's was performed. She felt so well that she returned to her senior citizen's dancing group, met an older gentlemen who proposed marriage, and returned to me, asking that her vaginal function be restored. Although you cannot always trust the patient's sexual aspirations, I would agree, in general, with the author's criterion for his selection in this regard.

Again, my congratulations to Drs. Langmade and Oliver for this excellent contribution. In this and other papers, Dr. Langmade presents a lifetime of experience with surgery for vaginal prolapse by both abdominal and vaginal routes and represents one of the country's foremost authorities in this type of surgical repair.

Dr. Jesse Rust, San Diego, California. I didn't hear you say anything about preventing stress incontinence. Do you put in a Kelly stitch in the anterior wall repair?

I wish to make one other comment. You mentioned the Mersiline mesh sacral colpopexy. We reserve that operation for the prolapsed normal-caliber vagina, with the vaginal repair done only for the voluminous prolapses.

Dr. William Graves, San Francisco, California. I'd like to thank Dr. Langmade for another surgical-technical contribution.

I'd like to ask, How many patients were successfully using vaginal pessaries during the period of this study, in contrast to the 24 unsuccessful pessary trials in this group of women with genital prolapse?

Dr. Paul Shively, San Francisco, California. I presented a similar paper at Salishan in 1968, and my series was small. In fact, it was so small that it was not in the

official GRAY JOURNAL but was recorded in the proceedings of the Pacific Coast Society. The study was on enterocele and partial colpocleisis. I think that partial colpocleisis is an excellent operation for a very small, select group of patients.

DR. LANGMADE (Closing). Dr. LeCocq, your discussion was flattering, and I appreciate very much your kind words, especially when you have had so much experience in vaginal surgery.

You are correct. The teaching of vaginal procedures these days leaves something to be desired in the medical school and county hospital settings. My thoughts are that the 65-year-old and over patients with prolapse now are seen as private patients under Medicare, and the older group is not seen in medical school and county hospital training centers. Further, other scientific programs fill more of the curriculum than do vaginal operations.

You are absolutely right in regard to draining of the perineum. We alternated our drains, and it did not seem to make a great deal of difference whether we drained them or not. We did not drain the vaginal vault in any case.

Foley catheters are considered to be old-fashioned today; however, none of them plugged up, and none of the patients seemed to mind too much about going home with a leg bag.

Dr. Rust, in regard to your question of management of stress incontinence, we did an anterior repair and were fortunate to have as many patients fall into the 85% good results that we would expect with an anterior repair. The only two patients with postoperative stress incontinence had stress incontinence prior to the surgery. Your remarks comparing the voluminous vaginal vault prolapse with the telescoping type are most interesting, and we know that you treat the bulging type with vaginal repair and the telescoping type with a Mersalene strip. You mentioned that we do do some vaginal procedures, and I have been trying to tell you that for years.

Dr. Graves, in regard to your question on the use of pessaries in these patients, we did try pessaries for 24 patients, and they did not tolerate them well. How many in general we see, I can't tell you exactly. However, we probably see three or four patients every month who are doing well with the pessary and would require no surgery.

Dr. Shively, I am sorry I didn't mention your contributions in the paper you presented in 1981. It was a small series, but it was a start, and most certainly we do appreciate your contribution.

A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance

Douglas Der Yuen, M.D.
Seattle, Washington

In an era when medical-legal suits and awards are escalating, a major metropolitan hospital in Seattle, Washington, has developed a plan to identify and effectively deal with problem physicians, improve patient care, and reduce medical liability for the institution. The Department of Obstetrics and Gynecology of The Swedish Hospital Medical Center formed a risk management committee that met regularly to monitor and evaluate clinical complications and questions of professional incompetence or physician impairment. A system for detecting such cases was established through liaison with other hospital peer review committees or any physician or nurse who was privy to specific information and willing to submit it in writing. Cases were carefully and objectively analyzed and evaluated as to their significance and severity. Single serious errors in case management were brought to the attention of the offending physician. Chronic offenders had privileges restricted or revoked with the approval of the hospital executive committee. This has resulted in improved medical care for patients and a reduction in medical-legal action against the hospital. (Am J Obstet Gynecol 1986;154:1206-10.)

Key words: Risk management, quality assurance, peer review

Professional liability is one of the most disturbing problems presently confronting both obstetricians-gynecologists and hospital administrators in the United States. Dramatic increases in the frequency of suits, astronomical awards to claimants, and skyrocketing malpractice insurance premiums are merely the tip of the iceberg as regards a crisis that is threatening the future of our specialty. Preoccupation with this problem is altering the pattern of obstetric practice in a manner not always to the patient's benefit, since physicians feel compelled to practice "defensive medicine" in response to the ever-present threat of litigation. Tests and procedures of questionably genuine indication further escalate medical costs. In addition, many experienced and competent physicians are finding it expedient or even necessary to drop their obstetric practices altogether.

Everyone currently facing this dilemma recognizes there is no immediate solution. Neither attorneys nor insurance carriers can be expected to resolve the problem, and a legislative solution in terms of tort reform is not likely to be forthcoming in the foreseeable future. Hence, in an attempt to reduce the number of poorly managed cases in its obstetrics unit and on its gynecologic service, The Swedish Hospital Medical Center, a major metropolitan hospital in Seattle, Washington, instituted an obstetrics-gynecology risk management committee to: (1) document instances of substandard care, (2) deal appropriately with poorly qualified physicians, and (3) ultimately improve the quality of patient care and reduce medical liability for both the medical staff and the institution.

The Swedish Hospital Medical Center is a 652-bed private teaching institution in Seattle, Washington; Swedish serves as the major metropolitan maternity center for Seattle, Washington, with approximately 3600 deliveries annually and more than 20,000 surgical procedures annually, of which more than 3500 are gynecologic. The central institution was founded 75 years ago as The Swedish Hospital, and, in 1978, the present title was adopted on merger with The Doctors Hospital, Seattle General Hospital, and Maynard Hospital, with the amalgamation of the respective medical staffs. Among these staff members were 75 obstetricians-gynecologists, nine practicing strictly gynecology, and 68 family practice physicians with obstetrics and gynecology privileges. Within this large group of practitioners there existed a wide spectrum of training, experience, and competence; some members are certified by their respective boards, and others are not. Because of this wide variance of skills and training, individual staff privileges are carefully reviewed annually by the chief of obstetrics and gynecology and the full-time department director and teaching coordinator.

The Swedish Hospital Medical Center is fortunate to have a full-time teaching administrative director for

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obstetrics and gynecology who is board-certified in both obstetrics and gynecology and clinical genetics. His position is supported by three full-time hospital-based associates: two perinatologists and a doubly trained internist-obstetrician. The hospital also employs a full-time neonatology staff. In addition to providing consultative support for the active staff, these physicians maintain a referral practice and provide a wide variety of reproductive laboratory support facilities.

The Swedish Hospital Medical Center also provides an important teaching function in the Seattle medical community, with four obstetrics and gynecology clinical residents from the University of Washington program (20% of the total staff) present at any given time, and providing obstetrics and gynecology clinical experience for the institution's own 20 family practice residents and family practice residents from Providence Hospital.

In 1982, after considering the rapidly worsening medical-legal climate, both locally and nationally, the elected chief of obstetrics and gynecology and the administrative director of obstetrics and gynecology expressed to the hospital administration their opinion that it was necessary to monitor obstetrics and gynecology practice in a structured, orderly fashion, in the hope of reducing mistakes of medical judgment and incidents of negligence. The administration responded with encouragement and assurance of support. Hence, with the consent and support of the obstetrics and gynecology and family practice staff, an obstetric-gynecologic peer review committee, known as the obstetrics and gynecology risk management committee, was formed. It was recognized that members of this committee should be carefully selected to discourage allegations of bias, conspiracy, or restraint of trade. Accordingly, a representative mix of respected practitioners in obstetrics and gynecology, including various premerger hospital staff members, different subspecialty groups, and family practitioners, was arranged to form a seven-person committee appointed by and including the chief and the director of the obstetrics and gynecology service.

Since its inception, the risk management committee has met regularly on a quarterly basis, and in emergency sessions, to consider and evaluate alleged physician impairment, single flagrant errors in clinical practice, or repeated episodes of substandard patient care. It was made known that the committee would accept input from other hospital practice committees, such as the obstetric mortality and morbidity committee, the operating room committee, the surgical tissue committee, and the quality assurance committee, as well as from any individual physician or nurse wishing to submit a complaint or evidence in writing. In each case, the initial task of the committee was to carefully and objectively determine the veracity of the charges by scrupulous review of the medical records and/or testimony of witnesses. It was considered vital to guard against any untrue charges that might have been maliciously motivated, as by jealousy, resentment, or overzealous competition for patients. Care was taken to eliminate differences in personality or in temperament as criteria for medical competence; in addition, "cookbook rigidity" of practice or technique was watched for as a possible basis for charges. It was always kept in mind that the absolute rights and wrongs of medicine may be too hard to define and are often subject to interpretation.

In many cases it was determined that no serious problem existed. With minor chronic problems or single errors, the committee made the physicians aware of the problems and worked constructively with the offenders to help them improve. Specifically, (1) interviews were always offered and sometimes insisted on with the physician in question; (2) written follow-up was sent, offering help—primarily suggesting more frequent use of consultation in difficult cases or problem areas and/or continuing medical education focusing on areas of demonstrated weaknesses.

When a single serious or recurrent problem was identified, the physician reviewed was asked to appear for a hearing before the committee. All business conducted by the committee was kept in strictest confidence. Complete factual minutes of all proceedings have been kept under lock and key, available only to the chief and the director. According to the hospital's legal counsel, these records are not subject to subpoena.

In accordance with hospital bylaws, the risk management committee could only recommend that action be taken by the executive committee, except in cases in which continued practice by a physician was deemed a clear danger to patients; then the chief of the department or the hospital chief of staff could exercise his authority to summarily suspend the physician's privileges, pending outcome of the investigation.

The hospital bylaws have subsequently been amended to permit greater flexibility by the risk management committee and departmental chief to restrict physicians' privileges in situations not warranting summary suspension of all privileges. Reports and summaries of the committee's recommendations were transmitted to the executive committee for definitive action, and, when necessary, to the hospital board of trustees and administration. All action taken strictly followed hospital bylaws' procedural codes so there would be no interference with the offending physician's right to due process.

It should be emphasized that without the wholehearted support of the hospital executive committee,

chief of staff, and administration, the existence and function of the risk management committee would not be possible. As the committee's effectiveness in surveillance became clear, it earned the respect and gratitude of the hospital attorneys and insurance carrier and ever-increasingly the respect of the medical staff as well. All involved members found that by eliminating unfit or professionally unqualified physicians from the staff, the collective medical-legal liability for the institution will be greatly reduced, and medical care will be improved, both by protecting patients from the actions of substandard or negligent doctors and by helping those doctors to improve their performances.

During its three years of existence, the risk management committee, with the support of the executive committee, has revoked full staff privileges of four chronic offenders in obstetrics and gynecology. In addition, three other physicians who were found to be clearly deficient in specific areas of practice underwent appropriate privilege restrictions.

Farmers Insurance Company, the liability carrier for the hospital, indicated that there has been an extremely low number of claims made against The Swedish Hospital Medical Center since the inception of the risk management committee. The company stated that they consider this particularly impressive in view of the size and volume of the obstetrics-gynecology service and the ever-increasing numbers of high-risk patients referred to the institution. Their records indicated that the departmental risk and quality control process has had some impact on reducing medical liability, although this is difficult to verify precisely. Comparisons of claims made against other hospitals in the area could not be validly made, since none are comparable in volume or numbers of high-risk patients cared for. Comparison of premerger to postmerger suits is not yet feasible. Three years of committee activity is too brief a period to evaluate adequately and in addition, in any specific cases, several years may elapse before a suit is generated. The Swedish Hospital Medical Center attorneys likewise feel that the activity of the risk management committee has had a positive influence in lowering hospital liability and have expressed their willingness to provide legal support whenever necessary. Last, the chief and director of obstetrics and gynecology both believe that since the risk management committee began to function, there has been a clear decline in the number of serious incidents brought to the attention of the obstetric mortality and morbidity committee at its monthly audits. This, they feel, is due to both an increased consciousness of the level of medical performance by the department as a whole and a general increased recourse to consultation in difficult cases.

Although there have been reports, particularly from California, of punitive suits brought against hospital peer review committees, to date there have been no legal actions brought against The Swedish Hospital Medical Center or the committee members as a result of peer review actions. It is felt that this is due largely to careful and strict documentation of all charges and actions and scrupulous adherence to hospital bylaws, as well as close maintenance of confidentiality. The committee members have always conducted themselves in good faith, have refrained from personal attacks, and have not discussed cases with other staff members, thereby reducing the likelihood of possible charges of libel or defamation. Although two suits were threatened, they were not pursued once the weight of the evidence against the defendant physicians was made apparent to their attorneys. The hospital insurance carriers have adamantly stated that they would back the committee with lawyers, as well as payment of any damages should they be incurred, an important consideration for any such activity. Members of the committee must have this sort of assurance of coverage by the institutional insurance carrier before assuming any activity.

In summary, there is suggestive evidence that frank and objective peer review in obstetrics and gynecology is possible. To avoid problems, investigation should be prompt, thorough, objective, and well documented. Due process, as defined by hospital bylaws, must be strictly observed. Scrupulous anonymity must be maintained. A supportive hospital administration is essential. Legal coverage by the institutional insurance carrier is mandatory. Above all, the committee must be fair, firm, and honorable.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Keith P. Russell, Los Angeles, California. With mounting professional liability pressures confronting our specialty, increased attention has been given to strategies designed to combat not only the rising numbers of litigations filed but also the soaring financial stresses. Among suggested approaches have been workmen's compensation type of insurance, trip insurance, policies containing greater deductible provisions, and other modifications. The problems of the various risks and malpractice dangers confronting the physician have been addressed by large numbers of medicolegal seminars, increased instruction at the resident education level, and the establishment of departments of professional liability within our major professional organizations. Three compartments of risk consideration have been approached: early detection, management, and prevention. Although the present article carries in the title "risk management," it is the latter category, "risk prevention," that the problem outlined in this excellent article addresses. By a method of rigid peer

review, the assessment of likely damage to the obstetric patient in this institution has been attacked by curtailing or terminating practice privileges of serious offenders within the department.

This matter of peer review has always been a perverse, thankless, and indeed challenging chore within our professional community. It is not a task that is commonly approached with great enthusiasm and is not one for which a reviewer can expect many bouquets. Many of our colleagues go to great lengths to avoid the responsibility. However, studies have repeatedly shown that true malpractice does exist. Many, if not most, suits are brought about by substandard professional care (unknowing, uncaring); in a certain small group these occurrences are repetitive. With this information in mind the author's institution has established a medical staff committee to deal with this specific problem.

This is a tedious process. To date the peer review committee, titled the obstetrics and gynecology risk management committee, has revoked full staff privileges of four chronic offenders in the three years of its existence. Two additional physicians have undergone privilege restriction. In my opinion, this is an excellent performance. Anyone who has dealt with the matter of terminating or eliminating privileges of a fellow professional knows that this entails much time, great soul-searching, and extensive legal activity for due process. In addition the peer review inevitably brings about certain interpersonal strains and stresses. I suspect that a particular problem in the Swedish experience has been the interfacing of family practice privileges within the obstetrics department. As one who had some peripheral involvement in the development of the American College of Obstetricians and Gynecologists—American Association of Family Practice document regarding this matter, I can assure this group that this is not always an easy relationship and usually requires rather constant monitoring.

Some questions inevitably arise on reviewing the present study. Although the author states that strict impartiality is maintained, this is frequently difficult to accomplish in an institution such as a hospital. How does the committee preserve confidentiality? What is the length of term of appointment of the members of the risk management committee? How many physician reviews actually came before the committee? In this day, when nurses consider themselves "patient advocates," what weight is given to nursing complaints and incident reports? Are all cesarean sections in the institution reviewed? Who reviews the reviewers (the committee), those paragons of practice?

In conclusion, I wish to compliment and congratulate the author and his colleagues on this report. These activities reflect a sound procedural concept and one that most obstetric services could and should emulate. The preliminary results speak for a very creditable program.

Dr. Gilbert A. Webb, San Francisco, California. I would like to commend the developing of this committee, but I do have one major objection. I think the motivation is inappropriate. I think our motivation should be to enhance the quality of care in an institution, not to protect ourselves from risk. Therefore, I would suggest changing the committee title to quality care committee rather than risk management committee.

Dr. William Hindle, Honolulu, Hawaii. At the Straub Clinic where I practice in Honolulu, we now number 110 physicians and own our own hospital, so we are highly motivated to control the liability and the risk in the hospital. We have had a similar sort of risk management program for about five years and it has been very successful. We do not have the same sort of results. Hopefully we have carefully selected physicians to join our hospital group, so we have not had to drop privileges or seriously restrict them, except for a few aging senior partners. But I rise to speak to the importance of this issue. As most of you know, I have the privilege of serving as the president of the Hawaiian Medical Association. I spend a lot of time talking about medical malpractice. If one studies the national medical liability trends, particularly as exemplified by obstetrics, it is apparent that the whole system of medical liability is going to collapse in the next few years. This is due to the cost, which is rapidly becoming unaffordable. The premiums are escalating at a rate that even the nation, much less individuals, cannot afford. The time to respond legally to adverse incidents has become so prolonged that it takes about two years for a case to actually be filed and more than six years before it is settled. This delay in the legal system does not serve anyone involved. And the liability caseloads are rising so rapidly in number that the courts are unable to handle them under the present system. It is essential that we physicians exert ourselves as the guardians of the quality of care and as those who determine the standards of practice. This must remain a professional decision, as brought out by Dr. Gilbert Webb and Dr. Keith Russell. Effective physician peer review is critically important. Otherwise, the standards of care will be set by the very aggressive judiciary system, the judges and the juries. They are actively doing this in the courts from their point of view of expansion of human rights and individual rights. The courts are taking away from the physicians the prerogative to set standards of care and standards of practice. So we physicians must exert ourselves in these traditionally professional areas.

By the nature of our practice specialty, we will always have adverse outcomes in a certain number of cases and a certain number of significant complications. These must be dealt with efficiently, effectively, and fairly to all parties involved. There are few cases of true medical malpractice, but the incidence gets distorted because most of the cases that do finally get through the courts and are judged in favor of the plaintiff, overwhelmingly are gross malpractice. However, such unfortunate cases are a very small percentage of the total number of suits that are filed. One approach to the future is for corporate liability responsibility on the hospital level with very strict control and active annual

review of privileges and credentialing specific for each operatice procedure. The critical essential of such a program is physician peer review no matter how difficult it may be to have physicians do this. If we physicians do not do this, it will be done to us.

E. Forrest Boyd, Los Angeles, California. We have been interested in this problem for quite awhile and I would like to bring up the subject of the patient advocate and the patient's perception of what happened. We have had a patient advocate, who was a former nursing supervisor in surgery, for several years in our hospital. We have about 130 total house staff and we are very heavily involved with unions and their type of personnel.

A few years ago, we developed this patient perception concept, and we now have the policy that any time there is an untoward incident or a patient complaint, this is evaluated, usually more by nurses than by doctors. We have several nurses in the Social Medicine Department who immediately contact the patient before they leave the hospital, find out what their perception is of what went wrong or why they were unhappy. It is surprising how many of these problems can be resolved before they even leave the hospital. While I think it is very important and essential that we police ourselves and our peers, I think that what we are being judged by primarily is the public perception of what happens. It may not be the truth, but nevertheless those are the criteria used. We are finding that this tactic is becoming extremely successful. Usually the doctors involved are very concerned and many times are at a loss as to what to do. There is an apparent barrier between the patient or the family and health providers. Many times it is a family-generated problem. But I would like to focus on the concept of patient perception in line with our peer review.

Dr. Der Yuen (Closing). To answer Dr. Russell's question, "How does the committee preserve confidentiality?" we implore each of the members of the committee not to discuss any committee activity with any person outside of the committee. All records are kept in a locked safe so there will be no inadvertent exposure of the minutes to non–committee members. As with any hospital, rumors obviously run rampant and there is lots of discussion among staff and nurses regarding doctors who are being scrutinized. I think it is vitally important not to discuss committee business outside the confines of the committee.

What is the length of term of appointment of members of this management committee? The term is indefinite. If a new chief is elected then he has the option or discretion to keep or dissolve the committee or name new members to the committee.

How many physicians faced committee review? Fourteen were reviewed but only four physicians had their privileges revoked. Three additional physicians had privileges restricted, leaving seven charges that either were dismissed as being groundless or were minor problems that could be dealt with by discussing the problem in a constructive manner.

The issue of jurisdictional disputes between physicians and nurses obviously is a sticky issue that is a sign of our times. Nurses are demanding an increased voice in medical decision-making. We have to be very careful that any complaints brought by nurses are not merely a matter of jurisdiction or a personality dispute. Nurses can be very competent observers, particularly in terms of the impaired physician, and sometimes are very astute observers of a physician's performance. We have to give weight to their complaints and comments. As with all complaints, they have to be scrutinized very carefully to make sure there are no motivating reasons other than medical competence or substandard medical care.

Who reviews the committee? Everyone is held accountable to a higher level and we are responsible to the medical executive committee of the hospital and the hospital administrator.

Dr. Webb brought up the question of whether our name should be quality care committee or risk management committee. I concur that the first priority is improvement of patient care. I think the medical-legal concern is secondary. We did not assume the name quality care committee because there is already a multidisciplinary committee of that name within our hospital that deals with broader areas of hospital practices and patient care. "Quality care" is probably a more appropriate term in terms of what we are striving to achieve.

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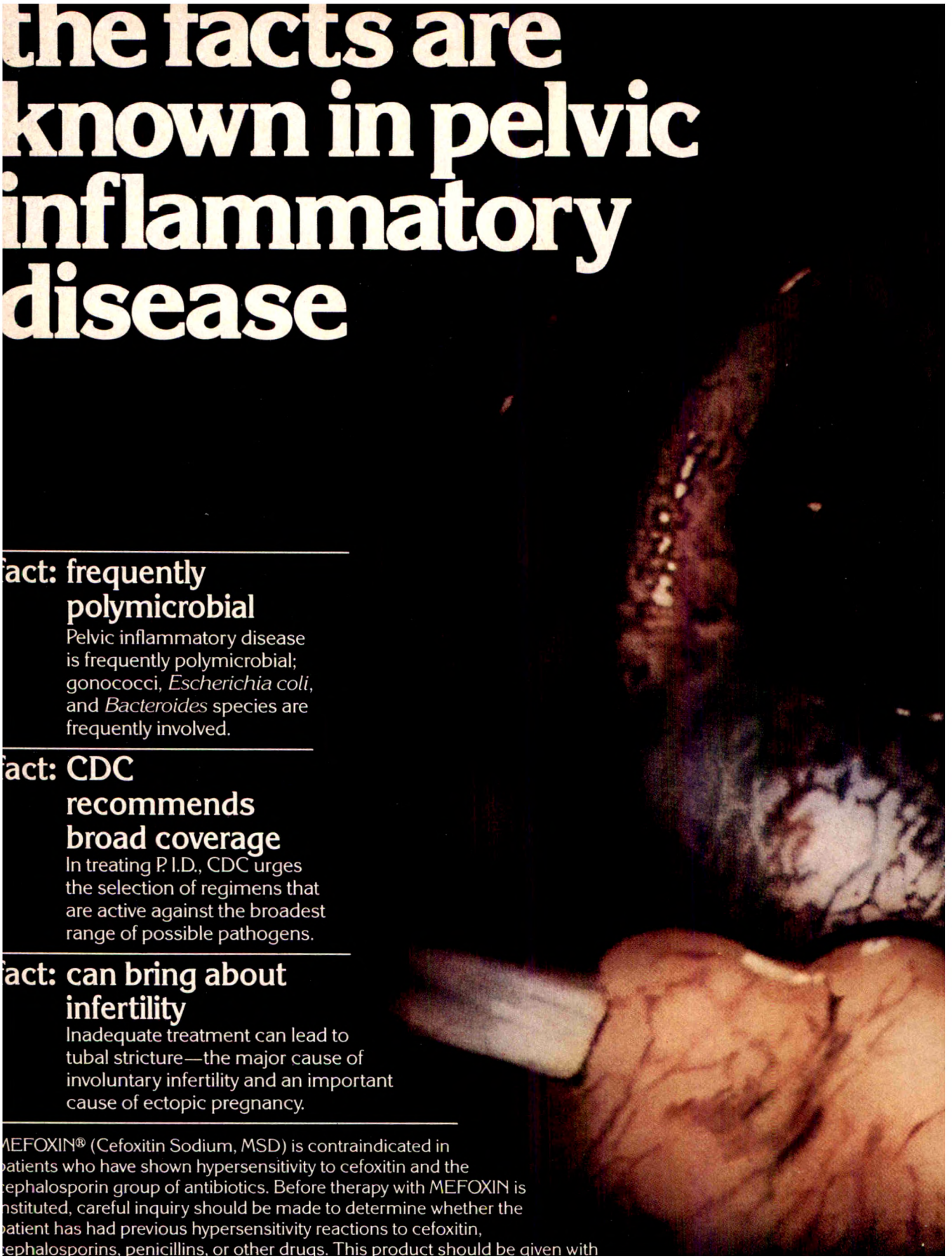
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GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae* (penicillinase and non-penicillinase producing).

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae* (penicillinase and non-penicillinase producing), *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.§

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,§ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Pseudomembranous colitis, from mild to life-threatening in severity, has been reported with virtually all antibiotics (including cephalosporins); therefore, it is important to consider its diagnosis when diarrhea develops in association with antibiotic use. Broad-spectrum antibiotics alter normal flora of colon and may permit overgrowth of clostridia; a toxin produced by *Clostridium difficile* is a primary cause of antibiotic-associated colitis. Mild cases may respond to drug discontinuance alone; in more severe cases, management may include sigmoidoscopy, appropriate bacteriological studies, fluid, electrolyte and protein supplementation, and use of a drug such as oral vancomycin; isolation of the patient may be advisable. Other causes of colitis should also be considered.

Precautions: *General*—Total daily dose should be reduced in patients with reduced urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur from usual doses. Prescribe with caution in patients with a history of gastrointestinal disease, particularly colitis. Prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures.

Drug Interactions—Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Drug/Laboratory Test Interactions—High concentrations (>100 mcg/mL) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. High concentrations may interfere with measurement of urinary 17-hydroxy-corticosteroids by the Porter-Silber reaction and produce false increases of modest degree in levels reported. A false-positive reaction for glucose in urine has been observed with CLINITEST II reagent tablets.

Carcinogenesis, Mutagenesis, Fertility Impairment—No long-term animal study has been performed on carcinogenic or mutagenic potential. Rat studies at approximately three times maximum recommended human dosage revealed no effects on fertility or mating ability.

Pregnancy Category B—Reproduction studies in rats and mice did not reveal teratogenic or fetal toxic effects, although fetal weights were slightly decreased. In rabbits, cefoxitin was associated with a high incidence of abortion and maternal death, neither considered teratogenic. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers—Excreted in human milk: Exercise caution.

Pediatric Use—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash (including exfoliative dermatitis), pruritus, eosinophilia, fever, and other allergic reactions including anaphylaxis. *Cardiovascular*—Hypotension. *Gastrointestinal*—Diarrhea, including documented pseudomembranous colitis during or after treatment, and, rarely, nausea and vomiting. *Blood*—Eosinophilia, leukopenia including granulocytopenia, neutropenia, anemia, including hemolytic anemia, thrombocytopenia, and bone marrow depression. A positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels and, rarely, acute renal failure.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent and in 10-gram bulk bottles.

§ *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*

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Modified retropubic cystourethropexy

John A. Goodno, Jr., M.D., and Thomas W. Powers, M.D.
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A variation of retropubic suspension of the urethrovesical junction was performed on 26 patients with urinary stress incontinence. Access to the retropubic space was through a low transverse muscle-splitting incision. Surgical technique consisted of securing a helical Prolene suture in the paraurethral fascia at each side of the bladder neck. Each suture was then passed through the abdominal wall fascia, exiting just above the symphysis pubis on either side of the midline, and tied to the opposite suture above the rectus fascia. The desired amount of elevation of the vesical neck was gauged by a Q-tip applicator inserted into the urethra just before the Prolene sutures were tied down. A cystotomy was performed to rule out any inadvertent penetration of the bladder by the nonabsorbable suture material, and the bladder was drained suprapubicly. The small number of cases and short follow-up times do not allow conclusions to be drawn at this time, but results to date have been encouraging. (Am J Obstet Gynecol 1986;154:1211-6.)

Key words: Urinary stress incontinence, cystourethropexy, retropubic

In our practice we were not satisfied with standard abdominal operations for urinary stress incontinence, as detailed in the recent excellent review article by Richardson and Ostergard.[1] We therefore began to study and evaluate alternative approaches. Noting that the modified Pereyra procedure[2] employed a *helical* nonabsorbable suture in the puboeurethral ligament to suspend the bladder neck to the rectus fascia, we developed a transabdominal modification for those patients in whom the abdominal route is preferred.

The concept of suspending the vesical neck from the rectus fascia is not new,[3] but the use of the helical suture in the paraurethral fascia represents an important modification, in our opinion.

We review the surgical technique and short-term results in 26 patients who underwent surgery between December, 1982, and April, 1985.

Material and methods

Patients ranged in age from 27 to 72 years. Preoperative assessment was primarily by history and physical examination. The history was detailed to rule out urgency incontinence, frequent small voidings, and nocturia, which would indicate bladder instability or a possible neurogenic bladder. Pelvic examination to determine the extent of rotational descent of the bladder neck, the hormonal status of the vaginal epithelium, the tone of the levator musculature, and neurologic status was performed.

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A catheterized urine specimen for residual and microscopic urinalysis was obtained. If residual urine volume was above 60 ml, urethrocystoscopic and urodynamic studies, including urethral calibration, were performed.

A preoperative stress test was performed when there was doubt as to the patient's history of urinary stress incontinence. If we were convinced of a valid history, together with supportive anatomic findings, an occasional patient in whom the stress test was negative underwent retropubic cystourethropexy. In this regard we are inclined to agree with Robertson (Robertson JR, M.D., personal communication, July, 1984) that some patients, under the tension of an office test, may artificially inhibit demonstrable urinary leakage by contracting all pelvic musculature.

Of the 26 patients, nine had preoperative urethrocystoscopic studies. Patients with stress urinary incontinence in whom there were indications for vaginal surgery were not included in this study. In some cases the final choice of operative route was not made until after the examination with the patient under anesthesia. The "Q-tip test"[4] often helped to gauge the degree of rotational descent of the vesical neck. There were no cases of secondary urinary incontinence in this study. Hysterectomy was performed in 20 of the 26 patients for coexisting indication.

All patients received 2 gm of Mefoxin intravenously 1 hour prior to surgery, and 2 gm of Mefoxin was given in the recovery room.

At surgery the patient was placed in the "frog leg" position so that the surgeon could insert two fingers into the vagina and elevate the paraurethral tissues during suture placement. A No. 22 Silastic Foley catheter was inserted into the bladder and inflated with 20 ml of water. Access to the operative area was generally

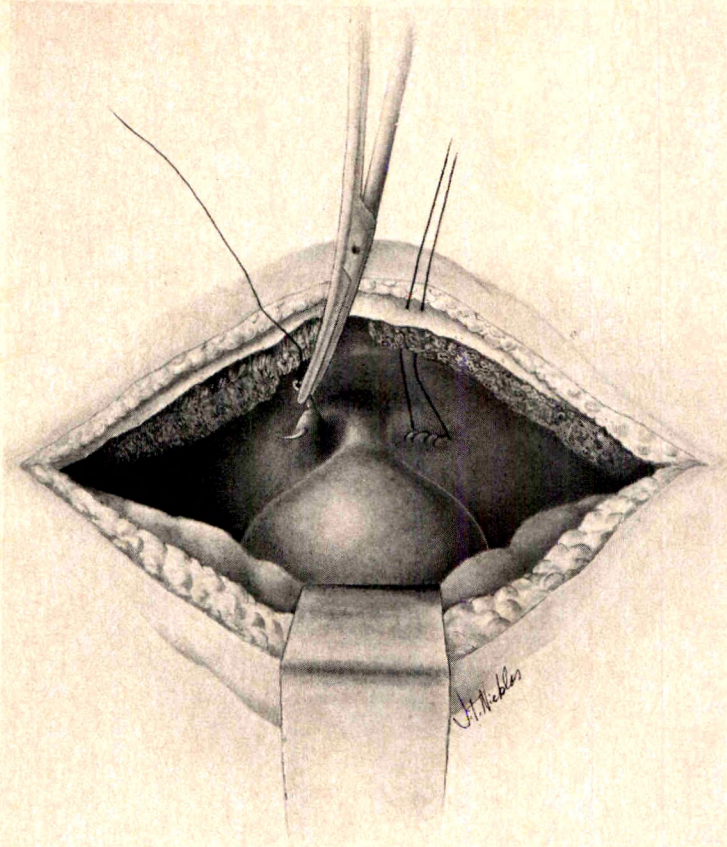


Fig. 1. Illustration of placement of helical suture.

through a Maylard incision, since we believe that the transverse muscle-splitting incision provides much better exposure to the retropubic space.

The paraurethral fatty tissue was bluntly dissected away, and a Babcock clamp was placed on the urethra just below the vesicourethral junction. Prior to placement of the paraurethral sutures, one ampule of indigo carmine dye was given intravenously. One helical Prolene suture was then placed on each side of the urethrovesical junction just inferolateral to the bladder neck. Four or five "bites" were taken through the full thickness of the paraurethral fascia. The axis of the helical suture was perpendicular to that of the urethra. The most medial "bite" of the suture was placed no closer inward than 1 cm from the urethra (Fig. 1).

The bladder dome was then opened by means of a small transverse incision, and the transurethral Foley catheter was removed from below. The bladder was inspected for any inadvertent suture penetration, and the urethral orifices and vesicourethral junction were examined. Traction was placed on the suspensory sutures at this point to assess the effectiveness of their placement. The two ends of each suture were then threaded through a large Mayo needle and passed through the rectus muscle and fascia about 1 to 2 cm above the pubic symphysis and 2 cm lateral to the midline on each side. A No. 14 Silastic Foley catheter was then brought into the retropubic space through a stab incision in the abdominal wall just below the skin incision and inserted into the bladder through the aforementioned cystotomy incision. The bladder was closed with two layers of 00 chromic catgut suture. The retropubic space was drained by a Jackson-Pratt suction drain exiting through a second stab incision just inferior to the abdominal incision but on the opposite side of the suprapubic catheter.

After the rectus fascia was closed, a sterile, lubricated Q-tip applicator was inserted into the urethra and the Prolene sutures were tied to each other over the rectus fascia. The degree to which the bladder neck was elevated was judged by the urethral axis. A urethral axis of 15 to 20 degrees below the horizontal axis was considered optimal in performing this step.

Postoperatively the suprapubic catheter was connected to gravity drainage for 48 to 72 hours. The patient was then allowed to plug the catheter, permitting the bladder to fill, and to attempt voiding if possible. Residual urine volumes were measured after each voiding or voiding attempt. When the urine residual was less than 2 to 3 ounces, the suprapubic catheter was removed.

Patients were generally discharged on the fifth post-

operative day. Those patients who were not voiding satisfactorily by this time were discharged with the suprapubic catheter and instructed to call us when the residual urine volume was less than 3 ounces. At this time they returned to the office for catheter removal. Urinary antibacterial agents were prescribed as long as the catheter remained in the bladder. The earliest a patient voided satisfactorily was the fourth postoperative day, and the longest voiding time was 15 days. The average voiding time was 7.5 days. The retropubic drain was removed on the third or fourth postoperative day because serosanguineous drainage was minimal by this time.

Analysis of results. Follow-up time in the group of 26 patients ranged from 4 months to 29 months postoperatively. No major complications were encountered. An occasional patient experienced dysuria and pyuria, which responded to appropriate antibacterial agents. Transient hematuria was frequently encountered, and the suprapubic catheter was irrigated with small quantities of sterile saline solution as necessary. As mentioned previously, the average number of days of postoperative bladder catheterization was 7.5 days, with a maximum of 15 days.

There were no cases of persistent or recurrent stress urinary incontinence during the follow-up period. Of the 26 patients who had both stress and urge incontinence prior to the surgery, three still reported urgency incontinence postoperatively. To date, postoperative evaluations with the "Q-tip test" have revealed excellent elevation of the previously sagging vesical neck, and there have been no cases of overcorrection with the resultant bladder outlet obstruction.

Comment

There are three salient features in our modification of the retropubic cystourethropexy. First, we used a helical monofilament nonabsorbable suture to obtain a more lasting and more secure purchase of the paraurethral fascia placed exactly at the urethrovesical junction. Stamey,[5] in his discussion of endoscopic suspension of the vesical neck, emphasizes the importance of suspending the fascia exactly at the vesical neck and avoiding suturing distally along the urethra, which may pull up the urethral meatus and flatten the urethra. Additionally, Pereyra and Lebherz[6] point out the ability of the helical suture to produce a ruffling, thickening, and pleating of the paraurethral tissues in order to prevent the suspensory sutures from pulling out at a later date.

Second, we believe that cystotomy is an important step, not only to rule out inadvertent penetration of the bladder by the nonabsorbable suture, but also to further assess accuracy of suture placement at the vesical neck. Although opening of the bladder sounds time-consuming to those not accustomed to this practice, it is simple and adds no more than 5 minutes to the operating time for the procedure.

Third, as a guideline for the extent of cystourethropexy, we believe that the amount of elevation of the vesical neck should be measured by some means in order to prevent undercorrection or overcorrection. Therefore we have employed a Q-tip applicator inserted into the urethra as a gauge to measure the urethral axis while the Prolene sutures are tied over the rectus fascia.

Regarding the preoperative workup, we are inclined to agree with Langmade and Oliver[7] that if the history does not indicate an urgency component to the incontinence, elaborate urodynamic studies are of confirmatory value only. In our study, urethrocystoscopy and urodynamic studies were performed only when there was a question of bladder instability or other urologic problems.

We have found suprapubic bladder drainage more comfortable for the patient, and it eliminates any guesswork regarding the time for catheter removal. Patients received printed instructions regarding catheter care in advance, as well as an informed discussion preoperatively. When patients were voiding consistently, with less than 3 ounces of residual urine, the suprapubic catheter was removed. Using this guideline, we have never had to reinsert a catheter.

Our use of the rectus muscle fascia, rather than the periosteum of the symphysis pubis or Cooper's ligaments, to suspend the bladder neck was motivated by the work of Pereyra and Lebherz[6] and Stamey,[5] who demonstrated the effectiveness of this type of suspensory suture. When operating vaginally for stress incontinence, we have employed the modified Pereyra procedure with gratifying results.

We agree with Mattingly and Davis[8] that patients must be followed up postoperatively for a minimum of 2 years to adequately evaluate the effectiveness of a surgical procedure performed for urinary stress incontinence. Since the average period of postoperative follow-up time in our study was 10 months, we are unable to draw conclusions as to the effectiveness of this technique, but we are encouraged by the results to date. We plan to continue to employ and study this technique when surgery by the abdominal route is indicated.

In an excellent review of stress incontinence in women, Graber[9] stated that at least 50 operations have been described for this condition. We have utilized many of the concepts previously worked out by others in developing our modifications of the abdominal cystourethropexy. The fact that so many surgical procedures have been devised for urinary stress incontinence seems to indicate that none is entirely without problems and that surgeons constantly need to reevaluate and modify their approach as new information and technology are forthcoming.

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Discussion

Dr. David Pent, Phoenix, Arizona. Dr. Jack Robertson, a pioneer in reawakening our interest in female urologic problems, once counted 135 surgical procedures for the correction of anatomic stress incontinence (Robertson, J. R., M.D., personal communication). It brings to mind the story of the professor of sociology who, in lecturing to his class on intercourse, announces that there are 93 different positions for having intercourse. He says that the first one is with the woman lying on her back and the man lying on top of her, facing her, at which point a French student in the back of the room says, "Ooh-la-la 94." In that sense, after hearing the presentation by Dr. Goodno and Dr. Powers, I guess we should say, "Ooh-la-la 136." Do we really need to have one more surgical procedure for correcting stress incontinence described? I think that the answer is yes, and the reason is that we are still ignorant and confused in our diagnosis and management of stress incontinence. Therefore every potential worthwhile contribution needs to be listened to, tried, and evaluated. Our specialty has failed to reach a consensus concerning the preoperative evaluation, the intraoperative surgical approach, or the postoperative evaluation. Preoperatively there are those who believe that every patient should have urodynamic testing and others who believe that the diagnosis can be made on clinical grounds, with urodynamic testing being reserved for a small subgroup. Intraoperatively there are those who believe that the successful cure of the problem is dependent on an abdominal procedure, and there are those who are equally vehement in insisting that restoration of the anatomy can be achieved only by the vaginal approach. Postoperatively we cannot even agree on what constitutes a cure, some authors accepting marked improvement in symptoms as evidence of a successful repair and others subjecting their patients to electronic monitoring to determine the loss of even small amounts of urine. This lack of agreement on what even constitutes a cure is one facet of the larger problem, in that although individual authors have tried to classify the degrees of incontinence, there is no generally accepted method of classification. Rational treatment has to be based on some system that takes into account the patient's age, her menopausal status, her weight and degree of obesity, her parity (or, in this age of cesarean sections, the number of vaginal deliveries might be a more valuable statistic), and, of course, the objective anatomic findings with regard to the urinary tract.

There is little, considering the 130-odd procedures already described, that is genuinely new in the surgical correction of stress incontinence, but Dr. Goodno and Dr. Powers are to be complimented on selecting certain features and bringing them together in one procedure. They have used the helical monofilament, nonabsorbable suture of the Pereyra procedure to solve the problem of the suture's tearing through attenuated fascia, which is thought to be the cause of many of the failed abdominal procedures. Other authors have addressed the problem in varying ways, such as putting in a series of sutures above the urethrovesical junction to help take some of the strain off the urethrovesical suture at the time of increased intra-abdominal pressure. Dr. Goodno and Dr. Powers also utilize a cystotomy to rule out the placement of the nonabsorbable sutures through the bladder mucosa and to allow confirmation of the effectiveness of the suture placement. Their Maylard incision affords excellent exposure, and obviously the better the exposure, the easier the procedure and the higher the success rate. Finally, the use of some objective form of measurement, in this case elevating the urethral axis to 15 to 20 degrees above the horizontal, has helped to avoid both overcorrection and undercorrection. As the article states, the number of patients is small, follow-up is short, and, in addition, we must remember that every new procedure, when initially reported, has a high success rate in the hands of its developer. Only when the procedure falls into the hands of the rest of us can we more accurately determine its true success rate.

Two questions come to mind. First, it was stated that this procedure was selected "for those patients in whom the abdominal route is preferred." In light of the encouraging results, has consideration been given to performing whatever vaginal procedures are indicated but forsaking the anterior colporrhaphy in favor of this new modified retropubic cystourethropexy? Second, a thoughtful observer of the incontinence problem, Edward McGuire, a urologist, writes that in 8 years of Bonney-Marshall testing, he has found only two women in whom urethral urinary loss could not be stopped by the maneuver. He goes on to state that the best position for the Bonney-Marshall test is with the patient in the upright position and notes that "objective examination of a standing patient by a crawling physician is difficult.

I personally resort to this unpleasant routine when a postoperative patient complains that she is still wet. I am uncertain as to whether this is the case because I feel guilty or vengeful. In any event, the practice is unsatisfactory in relieving either emotional state, usually serving only to amuse the nursing staff."[1]

In addition, Bhatia and Bergman,[2] using urodynamic measurements, have shown that the Bonney-Marshall stress test restores continence by causing extrinsic compression of the urethra and urethrovesical junction, and therefore the test cannot be relied on as a prognostic screening procedure. Indeed, Dr. Goodno and Dr. Powers report that there were patients in their series in whom the stress test was negative but in whom they did the cystourethropexy because they were "convinced of a valid history together with supportive anatomic findings." Does Dr. Goodno believe in the prognostic value of the stress test?

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Dr. Solon Barbis, Sacramento, California. It seems that this procedure is formidable. What is the average length of operating time required to complete this operation?

Dr. Duncan Neilson, Portland, Oregon. I am interested in the semiquantitative measurement of the urethral angle with the use of the Q-tip to determine the final position of the bladder. The question I have is, Does the position of the legs affect this measurement? I gather that these patients were in the frog-leg position during the procedure, but if stirrups are used or a different position is used, is that angle measurement the same?

Dr. Goodno (Closing). Thank you, Dr. Pent, for your humorous and informative discussion and some very good points as to the variety of surgical procedures. In answer to the question about whether we do some procedures vaginally, generally we treated those patients who had obvious genital prolapse and big cystoceles from below, and we used the Pereyra procedure in those cases. The patients whom we treated had anatomic incontinence, but they did not have the marked prolapses some of those slides showed. In regard to the Marshall-Marchetti test, I don't think that it's greatly reliable—or prognostically valuable, either—and I think that Jack Robertson has suggested that perhaps we give women a couple of Pyridium tablets, have them wear a minipad around for a few hours, and then report back to us on the amount of staining of their perineal pad. We're considering doing this, both preoperatively and postoperatively, to objectively assess urinary incontinence.

Dr. Hibbard has commented about opening the bladder and inserting a finger in the urethra. We have done that from above. We've put our finger right down through the vesical neck, and it's surprising how often we find a patulous vesical neck. Then, although we haven't placed a suture doing that, we have then pulled up on the suture we have already placed to see how well it elevates the vesical neck.

As far as the formidability of the operation—it really isn't. It's very simple—opening and closing the bladder takes about 5 minutes. The entire procedure takes an hour or less. In answer to the question of whether the position of the legs affects the Q-tip test, we always have the table parallel to the floor when we measure that. As far as the patient's being in a frog-leg position or in stirrups, I haven't observed any great change.

Tubal conservation in ectopic pregnancy: A study of 200 cases

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There have been 1152 cases of ectopic pregnancy at Kaiser Permanente Hospital from 1953 to 1980. In 200 cases the involved tube was preserved with no surgical complications. There have been 122 subsequent pregnancies, 24 of which were repeat ectopic pregnancies. Twelve were in the involved tube and 12 were in the opposite tube, demonstrating conclusively that both tubes are at equal risk of ectopic pregnancy. Conserving the involved tube does not increase the incidence of ectopic pregnancy but does increase the chances for intrauterine pregnancy in this subfertile population. (Am J Obstet Gynecol 1986;154:1216-21.)

Key words: Pregnancy, ectopic; tubal conservation

The lethal nature of hemorrhage from ruptured ectopic pregnancy was reported in 1876 by Parry and Lea.[1] This series of 529 cases, with a mortality rate of 69%, prompted Lawson Tait,[2] the "father" of gynecologic surgery, to recommend salpingectomy as an emergency life-saving procedure in the treatment of ectopic pregnancy. He reported 38 cases in 1888, with only two maternal deaths. Complete salpingectomy, as soon as the diagnosis is made, has continued to be the conventional management of tubal pregnancy for the past 100 years.

About 15% of patients with ectopic pregnancy will have a repeat ectopic pregnancy in the residual tube, and removal of that tube results in sterility. Many of these patients requested that the tube be conserved to preserve their fertility. These cases have demonstrated the feasibility and safety of tubal conservation.

Important to an understanding of ectopic pregnancy and relevant to conservation of the involved tube is a classic report from Helsinki, Finland, in 1955, by J. J. Lund.[3] The report is summarized in Table I and reveals the natural history of ectopic pregnancy. Three hundred eighty-seven hospital admissions for ectopic pregnancy were studied. Fifty-five percent of the patients were observed to have subacute ectopic pregnancies. Forty-two percent of these patients had a recurrent hemorrhage, demonstrating the episodic nature of ectopic pregnancy, and required emergency operation. One third of the entire series had no further symptoms.

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Table I. Summary of cases of ectopic pregnancy reported by Lund, Helsinki, Finland, 1955

Total patients admitted with ectopic gestation	387	
Acute, requiring operation	167	(45%)
Subacute, patient observed	220	(55%)
Later required operation	92	(42%)
Medically resolved	128	(33%)

Table II. Summary of subsequent pregnancies up to menopause after ectopic pregnancy, reported by Lund, Helsinki, Finland, 1955

Treatment	*Intrauterine (%)*	*Repeat ectopic (%)*
Surgically treated (tube removed)	45	16
Medically treated (tube conserved)	45	15

Their pregnancy tests became negative, their masses resolved, and after averaging more than a month in the hospital, they were discharged. All patients were followed up through the menopause. Subsequent pregnancies are shown in Table II. Forty-five percent of both patients with and without operation had intrauterine pregnancies. Sixteen percent of the group with operation and 15% of the group without operation had repeat ectopic pregnancies. The cases without operation represent conservation of the involved tube.

Table III is a compilation of cases of tubal conservation in 1967, which shows that tubal patency is maintained and fertility is preserved after tubal conservation. Subsequent pregnancies were predominantly intrauterine. The repeat ectopic pregnancy rate was 15%, which was the same as that reported by Lund. Preservation of the involved tube in ectopic pregnancy is

Table III. Tubal conservation in ectopic pregnancy: Fertility after preservation of involved tube, 1967

Author	*No. of cases*	*Uterine pregnancy*	*Ectopic pregnancy*	*Tubes patent by HSG*
Skulj	45	23	?	86%
Matthaei	87	?	?	72%
Wexler	45	15	2	?
Lark	42	24	3	?
Tompkins	17	?	3	?
Stromme	45	32	7	?
Hallatt	87	25	5	?
Total	352	119	21	

HSG = Hysterosalpingography.

Table IV. Tubal conservation in ectopic pregnancy (n = 200), 1953 to 1980, in a total of 1152 cases

Location in tube	*Tube removed (%)*	*Tube conserved (%)*
Inner one third	11	15
Middle one third	31	28
Outer one third	58	57

Table V. Tubal conservation in ectopic pregnancy: Location of subsequent pregnancies

Subsequent pregnancies	*Two tubes*	*Only tube*
Uterine	98	10
Ectopic	24	5
Same side	12	
Other side	12	

Conservation with other tube present, n = 174; conservation of only tube, n = 26.

now regarded as an acceptable procedure and should be considered when fertility is desired by the patient. The purpose of this paper is to analyze the results of conserving the involved tube in 200 cases of ectopic pregnancy at the Kaiser Permanente Hospital in Los Angeles from 1953 to 1980.

Material and results

The Kaiser Permanente Hospital in Los Angeles provides prepaid comprehensive medical and surgical care for a representative cross section of the Southern California population. Ectopic pregnancy is managed by the entire staff of the Department of Obstetrics and Gynecology as an emergency surgical condition. The surgical procedure is determined by the personal preference of the staff person on duty. Some of the staff are reluctant to conserve a tube damaged by ectopic pregnancy. Those with a special interest in fertility are more likely to conserve the involved tube. Some of the staff will base their decision on the results of a tubal patency test at the time of operation or on the gross appearance of the contralateral tube.

Since the hospital opened, in 1953, until 1980, there have been 1152 cases of ectopic pregnancy requiring operation. In 200 cases or 17%, the involved tube was not removed. These 200 cases are felt to be representative of the entire series, as the location of the ectopic gestation was essentially the same as in the cases with salpingectomy, as shown in Table IV. Fifteen percent were in the proximal one third of the tube, 28% were in the middle one third, and 57% were in the outer one third of the tube. Subsequent pregnancies are shown in Table V. In 26 cases, the involved tube was the only tube and fertility was more compromised than in the patients with two tubes. While the numbers are too small to be statistically significant, pregnancies after involvement of the inner one third of the tube were almost as likely to be ectopic as intrauterine. Pregnancies after an involvement of the middle and outer portions of the tube were four times as likely to be uterine as ectopic. There were no surgical complications in the 200 cases and no patient required further surgical procedure to control bleeding. There were, however, many cases where tubal conservation was attempted, but operation terminated in a salpingectomy to control persistent bleeding. No special techniques or departures from standard gynecologic surgical procedures were used in any of the cases.

There has been a significant change in the findings in ectopic pregnancy in the last few years, as shown in Table VI. The findings in the 1152 cases are compared with those in the 226 cases occurring in the 3 years from 1980 to 1983. There has been a doubling in incidence of ectopic pregnancy in terms of deliveries. Ectopic pregnancy is occurring at a younger age. There are now twice as many cases after tubal surgery as before. Twelve percent of all ectopic gestations are now associated with the use of an intrauterine contraceptive device. This figure is directly related to the number of women using the intrauterine contraceptive device for

Table VI. Tubal conservation in ectopic pregnancy: Current changes in findings in ectopic pregnancy

	1953-1980	*1980-1983*
Total ectopic pregnancies	1152	226
Incidence per number of deliveries	1/111	1/58
Average age of patient (yr)	30	28
Prior tubal operation (%)	5	10
IUD associated	0%-16%/yr	12%

IUD = Intrauterine contraceptive device.

Table VII. Tubal conservation in ectopic pregnancy: Factors leading to early diagnosis and conservative management

	1953-1980 (%)	*1980-1983 (%)*
Pregnancy test positive	52	95
Ultrasound	2	23
Laparoscopy	5	18
No hemoperitoneum	5	15
Involved tube conserved	17	26

contraception. Table VII lists some of the factors that lead to an early diagnosis and enhance conservative management. The use of the very sensitive and specific β-subunit of human chorionic gonadotropin pregnancy test as a screening procedure in all high-risk patients identifies candidates for ultrasound and laparoscopy to aid in the diagnosis before rupture occurs. In the last 3 years, ultrasound was used in 23% and laparoscopy was used in 18% of all ectopic gestations. That the diagnosis is now being made early is documented by the finding of no hemoperitoneum in 15% of all ectopic pregnancies. The involved tube is now conserved in 26% of all ectopic gestations. Follow-up of 72% of these 200 cases indicates that future fertility favors uterine pregnancy over repeat ectopic pregnancy in both the involved tube and in the contralateral tube. Eighty percent of subsequent pregnancies were uterine pregnancies, and 20% were repeat ectopic pregnancies. It is significant that of the 24 repeat ectopic gestations, one half were in the involved tube and one half were in the contralateral tube. This follow-up is a crude evaluation of fertility, since the number of patients using contraception and not desiring or attempting pregnancy was not determined. Of the 1152 patients with ectopic pregnancy, 20% were infertile before the ectopic pregnancy.

Comment

The cause of ectopic pregnancy is chronic damage to the endosalpinx by prior infection or operation. There is fusion of the plicae of the endosalpinx, creating pockets that entrap the passively migrating zygote. On the sixth day after fertilization, the blastocyst will implant by trophoblastic invasion of adjacent maternal tissue. In the uterus, the maternal blood vessels are protected by decidua. In the tube, there is no decidua to protect the maternal blood vessels, so hemorrhage occurs very early in the pregnancy. Aggressive implantation and trophoblastic invasion and hemorrhage can continue for 12 weeks as long as there are viable villi. The damage to the tube from hemorrhage and extravasation appears to be much more destructive to the tube than it actually is. The damage is repaired physiologically by absorption of blood and regeneration of the endosalpinx, restoring tubal patency. This has been repeatedly observed at subsequent operation, where the involved tube is found to be anatomically normal. The anatomy is also restored by tubal peristalsis and ciliary action, whether the tubal defect is closed surgically or left open, as advocated by Pendleton Tompkins.[4] The dynamic physiology of the fallopian tube is as responsible for the success of tubal conservation as is the surgical procedure.

It is still too early to evaluate the role of atraumatic operation in ectopic pregnancy, but the preliminary reports do indicate a higher intrauterine pregnancy rate and a lower repeat ectopic pregnancy rate.[5-7] The inherent fact of ectopic pregnancy is that both tubes are in equal jeopardy of recurrent ectopic pregnancy. This is due to damage from a prior infection that involved the entire tube and that cannot be repaired surgically. The patient can be told that should she become pregnant, the pregnancy is more than four times as likely to be in the uterus as it is to be in the tube.

There have been several reports[8] of recurrent hemorrhage requiring operation after salpingostomy or the procedure of "milking" the pregnancy out the fimbriated end of the tube. If viable villi are left in the tube, then a subsequent hemorrhage may occur. The average gestational age of an ectopic pregnancy at operation is 6 weeks. The products of conception can be overlooked or obscured by the hemorrhage and blood clots. If one is not certain that the entire pregnancy has been enucleated, then one should evaluate the patient with serial determinations of the β-subunit of human chorionic gonadotropin which should decrease to negative in 2 weeks[9] unless viable villi persist or a second pregnancy is present.

If inspection of the ovaries reveals more than one corpus luteum of pregnancy, then one can expect to find another pregnancy, which may be intrauterine or ectopic.

While ultrasound is now used in 23% of all ectopic pregnancies, its greatest value is in identifying an intrauterine pregnancy, thus ruling out an ectopic pregnancy, except for the rare case of combined pregnancy. The ectopic gestation itself is rarely identified by ul-

trasound since the gestation is usually too early and is frequently distorted by hemorrhage disrupting the gestational sac.

Laparoscopy has replaced the diagnostic laparotomy in the diagnosis of ectopic pregnancy. The early unruptured ectopic pregnancy without hemoperitoneum can be diagnosed only by laparoscopy or laparotomy. These patients are ideal candidates for tubal conservation as they are stable and the tubal anatomy is not excessively distorted by hemorrhage and rupture.

Salpingostomy is best performed by using the unipolar cutting current to incise the tube and expose the implantation site. The gestational sac is enucleated by blunt dissection and bleeding points are cauterized with the bipolar thumb forceps. It is usually necessary to ligate the tubal artery that parallels the tube in the mesenteric border proximal and distal to the pregnancy. Bleeding points are best identified by irrigation with lactated Ringer's solution. The serosa is approximated with fine polyglycolic or polyglactic sutures as any raw area will result in adhesions. If bleeding cannot be controlled, then resection of the involved segment, rather than salpingectomy, is indicated to conserve the tube. This permits the possibility of later reanastomosis if indicated and desired. Should the patient not desire further pregnancies, then removal of only the involved portion of the tube is indicated. Partial salpingectomy does not increase the incidence of ectopic pregnancy.

The fertility of the patient with ectopic pregnancy is compromised, and the removal of the involved tube may further diminish her fertility. This study shows that the involved tube can be safely conserved and that subsequent pregnancies are predominantly uterine. It is logical to assume that two tubes favoring uterine pregnancy are better than one tube to enhance fertility. It is therefore recommended that the involved tube in ectopic pregnancy be conserved in the patient who desires a successful pregnancy.

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Discussion

Dr. Arthur M. McCausland, Sacramento, California. This article by Dr. Hallatt has great clinical significance, especially today.

In the past, the difficulty was just in making the diagnosis of an ectopic pregnancy before it ruptured. Today the problem is how to manage an ectopic pregnancy diagnosed at a very early stage. Our ability to diagnose an early tubal gestation through the use of the β-subunit of human chorionic gonadotropin, ultrasound, and laparoscopy has given us the option of a more conservative approach. This approach raises new questions.

First, does conserving the involved tube increase the risk of a repeat ectopic gestation, and second, does it enhance the chance for a future intrauterine pregnancy? Dr. Hallatt addressed these questions and has found that a conservative approach gives the best overall results.

A review of the world's literature confirms these findings. Schenker[1] reviewed 24 articles addressing similar questions. He also concluded that tubal conservation increases the number of future intrauterine pregnancies and does not increase the risk of repeat ectopic pregnancies in the involved tube over the contralateral tube.

Another statistic presented in Dr. Hallatt's paper was that if an ectopic pregnancy was found in the inner one third of the tube, the next pregnancy was as likely to be a repeat ectopic pregnancy as an intrauterine pregnancy. This 50% rate of repeat ectopic gestation is in contrast to a 15% repeat rate if the original ectopic pregnancy was found in the middle or distal portion of the tube. This is interesting because in my study[2, 3] of ectopic gestations following tubal injury, it was found that the proximal portion of the oviduct responds differently to injury than does the middle or distal portion of the tube. Tubal activation instead of fibrosis is seen in the proximal segment after injury, whether from operation or infection. This is why salpingitis isthmica nodosa is usually seen only in the proximal oviduct although the entire tube is usually infected. Therefore, the pathologic process causing partial tubal occlusion in the proximal oviduct might be expected to be more extensive than in the middle and distal portions. This could explain why ectopic pregnancy recurs more often in the proximal oviduct.

Dr. Hallatt mentioned that in Finland a significant number of patients with ectopic pregnancies were observed without operation. This nonsurgical management awaiting a tubal abortion was fascinating to me. Evidently, the same approach has been used in China. I do not know whether to call this treatment radical or conservative but I guess if a patient is lucky enough to spontaneously abort the ectopic pregnancy out the end of the tube without operation, the ultimate in tubal conservation has occurred. However, the hospital uti-

lization review committee may not understand the weeks of prolonged hospitalization.

In one case, a woman had an intrauterine contraceptive device and her menstrual period was late. The human chorionic gonadotropin was positive 6600. Her husband, an outstanding radiologist, found on ultrasound examination a 3 to 4 cm complex left adnexal mass and no intrauterine pregnancy. Since she was asymptomatic, he wanted me to follow up with serial human chorionic gonadotropin, hemoglobin, and daily ultrasound evaluations. Reluctantly I agreed and within 3 days, the adnexal mass disappeared. Cul-de-sac fluid was then present and subsequently resorbed. The hemoglobin was stable and the human chorionic gonadotropin dropped to zero. I cannot recommend this treatment but it certainly was thought-provoking.

I enjoyed reviewing Dr. Hallatt's article and have only two questions. First, was salpingostomy the only type of conservative operation done? Were any manual tubal abortions or segmental resections with anastomosis performed? Second, since there is a 50% rate of repeat ectopic gestation when the original one involves the proximal oviduct, should a salpingectomy be considered over a conservative approach in this situation, especially if the contralateral tube and ovary appear normal?

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DR. LEROY CASPERSEN, Portland, Oregon. Dr. Hallatt, how many patients underwent reoperation because of persistence of pregnancy symptoms or bleeding?

We have been performing salpingostomy, when feasible, for some time but have had three or four cases where we found it necessary to reoperate because of failure to remove all the gestational tissue.

DR. CLIFFORD CURTIS, Redwood City, California. During a 6-month period I personally managed six cases of tubal pregnancy; four patients were able to achieve a term pregnancy subsequent to conservative management and two of the patients, specifically Cases 4 and 6, had the misfortune of having two consecutive ectopic gestations. In Case 4 a salpingectomy was performed 5 months before I examined her. The pregnancy was in the infundibulum and was expelled at operation. She again became pregnant 5 months later and the pregnancy went to term. In Case 6, the first ectopic gestation was managed by a linear salpingotomy. Nine months later she had another ectopic pregnancy that was expelled. Eight months later she became pregnant again and this pregnancy went to term. We had, as has been mentioned, a bad local experience with hemorrhage and persistence of ectopic gestation after exploratory laparotomy at our hospital and, corroborated by the literature, decided that we had to do something else. Now fairly routinely, when we perform a salpingotomy or salpingostomy, we will ligate the mesosalpinx and put sutures through each of the arcades that are available. In the last 2 years, no doctor in our hospital has had to reoperate to repair a bleeding tube or remove a persistent ectopic trophoblast.

DR. JOHN MOLITOR, Fullerton, California. I am the chairman of the Bio-Ethics Committee at the Catholic Hospital at which I work. We have a new dilemma now with tubal pregnancies. Salpingectomy has been viewed as removal of an abnormal organ, and the termination of the pregnancy then was a "secondary, unintended effect," or the so-called principle of double effect. Some people now view salpingotomy as a direct attack on the fetus, which is, of course, morally illicit, according to church doctrines.

I feel that in most of these cases the fetus is already dead, even in the very early cases, even perhaps with no hemoperitoneum due to abruptio placentae.

My question for Dr. Hallatt is based on his broad experience with tubal pregnancy. How does he feel about the viability of the fetus at the time of salpingotomy? If most of them are already dead, then we have resolved our dilemma. If not, then we still have to face it.

DR. HALLETT (Closing). In answer to Dr. McCausland's question regarding whether salpingostomy was the only procedure in conserving the tube, actually, nature has done the salpingostomy, or salpingotomy, on the majority of these patients, and all we had to do was try to repair the damage. Fortunately, the dynamic nature of the tube is such that it repairs the damage that has been done. We must atraumatically accomplish hemostasis and approximate serosa to serosa, minimizing as much raw area as possible. The tube will reanastomose and reconstitute itself. It has been demonstrated that the endosalpinx can regenerate and span an area of 1 inch.

The second question was why ectopic gestations recur more frequently in the proximal portion of the tube. The proximal portion of the tube is different anatomically and physiologically from the outer two thirds of the tube. Whether one should remove it because of a 50% chance of recurrent ectopic gestation is a consideration. Many patients would desire a 50% chance of an intrauterine pregnancy. This compares favorably with that of in vitro fertilization, so I would conserve the tube. I hope to be able to accumulate enough cases to document this fact statistically.

Dr. Caspersen, none of the 200 patients had to undergo reoperation. There was no subsequent hemorrhage. As I mentioned, there have been several reports of subsequent hemorrhage requiring operation because the ectopic gestation was not completely removed.

Dr. Molitor, one third of all embryos are necrotic at the time of operation for ectopic pregnancy. The other two thirds are potentially viable but severely compromised and are destined to "self-destruct." Recurrent hemorrhage is the nature of the disease.

Attempts to transplant the gestational sac into the uterus, by myself and others, have been unsuccessful.

Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention

William M. Petty, M.D., Richard O. Lowy, M.D., and Albert A. Oyama, M.D.
Portland, Oregon

Abdominal hysterectomy after treatment of cervical cancer by radiation therapy is associated with a significant rate of postoperative vesicovaginal fistulas. In this series, five patients with invasive cancer of the cervix treated by radiation therapy developed new cervical or uterine neoplasms 1 to 27 years after treatment. All underwent abdominal hysterectomy without postoperative fistula formation. Success is attributed to cautious surgical technique and to the use of the omental pedicle graft to bring new vascularity to the vaginal apex and bladder base. The technique of forming an omental pedicle graft is described. (AM J OBSTET GYNECOL 1986;154:1222-6.)

Key words: Cervical cancer, radiation therapy, abdominal hysterectomy, omental graft

Uterine sarcomas, endometrial cancer, and other pathologic processes may occur in the retained uterus of a woman previously treated for cervical cancer by radiation therapy. Operative management of these problems is difficult and is associated with a high incidence of postoperative vesicovaginal fistulas. Radical abdominal hysterectomy is associated with a 17% to 53% incidence of postoperative fistulas in several small series.[1-3] Graham and Goplerud[2] report that 3 of 13 patients (23%) developed urinary fistulas after total abdominal hysterectomy. Six additional patients underwent total abdominal hysterectomy with omentopexy, and none of these patients developed fistulas.[2]

Direct attachment of the omentum to the vaginal apex may cause traction on the stomach, thereby giving rise to chronic epigastric discomfort.[4] Operative techniques have been developed to lengthen the omentum while preserving its blood supply.[4,5] These techniques allow a vascular omental pedicle to be brought down to the vaginal apex and bladder base without traction. These techniques are also useful in repairing radiation therapy–related vesicovaginal fistulas.[6,7]

Material and methods

From 1981 to the present time, five patients were treated for new, clinically localized cervical or uterine disease. All had had previous invasive carcinoma of the cervix that had been treated with radiation therapy. The original radiation therapy treatment records were available for four of the five patients. They were re-

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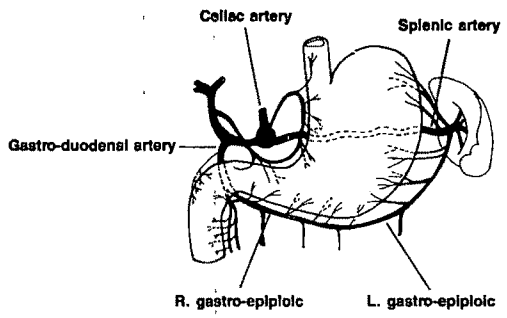


Fig. 1. The omental blood vessels arise from the right and left gastroepiploic arteries. The right gastroepiploic artery originates from the gastroduodenal artery, which comes from the common hepatic artery and ultimately the celiac artery. The left gastroepiploic artery comes from the splenic artery, which is also a branch of the celiac artery.

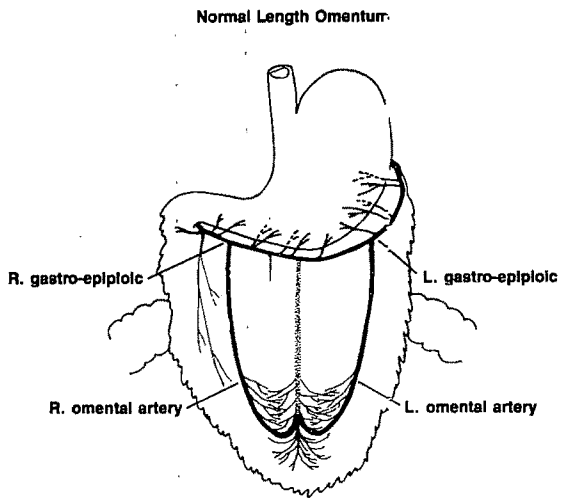


Fig. 2. The omental arteries are, from *left* to *right*, the accessory omental artery, the right omental artery, a variable middle omental artery, and the left omental artery.

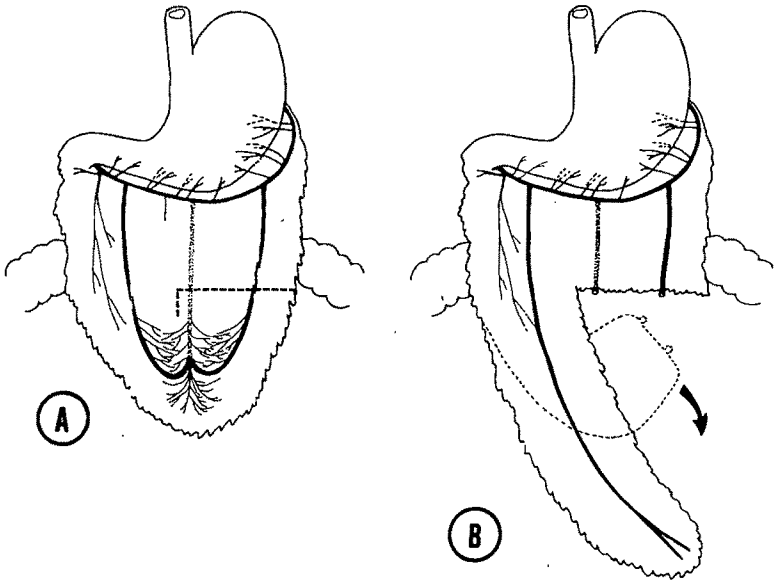


Fig. 3. *A*, The normal-length omentum is lengthened by partially transecting the omentum in reversed J configuration. *B*, This mobilized omentum is unfolded for attachment to the vaginal apex.

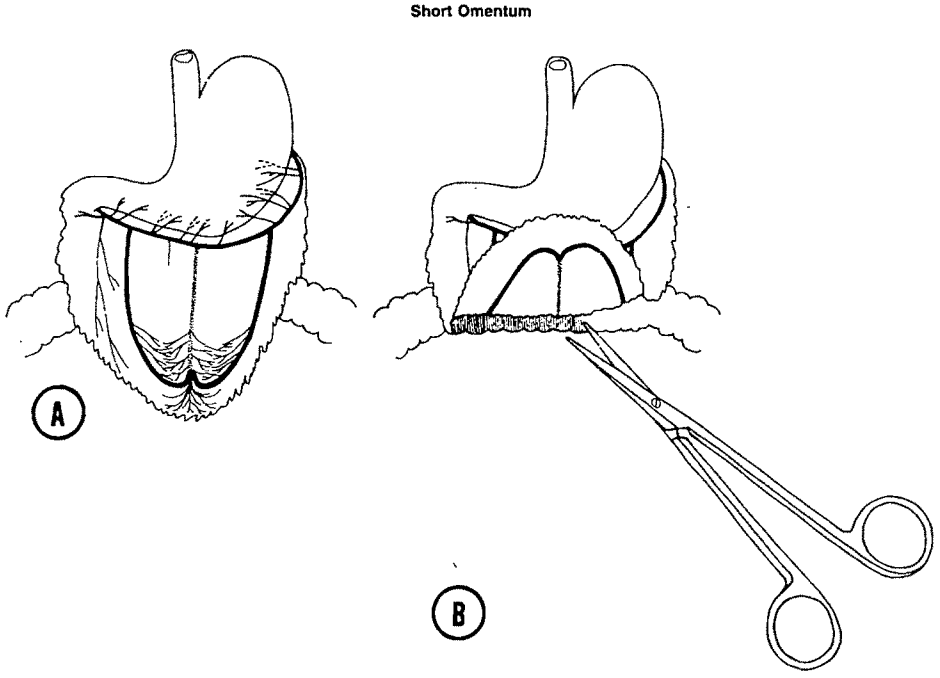


Fig. 4. *A*, Short omentum. *B*, Elevation of omentum exposes peritoneal attachment to transverse colon.

viewed by one of us (R. O. L.) and were found to conform to standard treatment.

In each case the new abnormality differed from the original cervical cancer. Slides of three of the five original and all of the new disease processes were available and were reviewed by one of us (A. A. O.).

Patient clinical characteristics are summarized in Table I. The hospital course and outcome for each patient are summarized in Table II.

All hysterectomies were performed through midline incisions. Appropriate staging procedures were performed, including peritoneal cell washes, lymph node biopsies, and other biopsies. Hysterectomy technique was meticulous in order to prevent bladder injury. Antibiotics, usually a cephalosporin with or without an aminoglycoside, were used prophylactically for 48 hours.

Patient E. M. had an omentum that was long enough

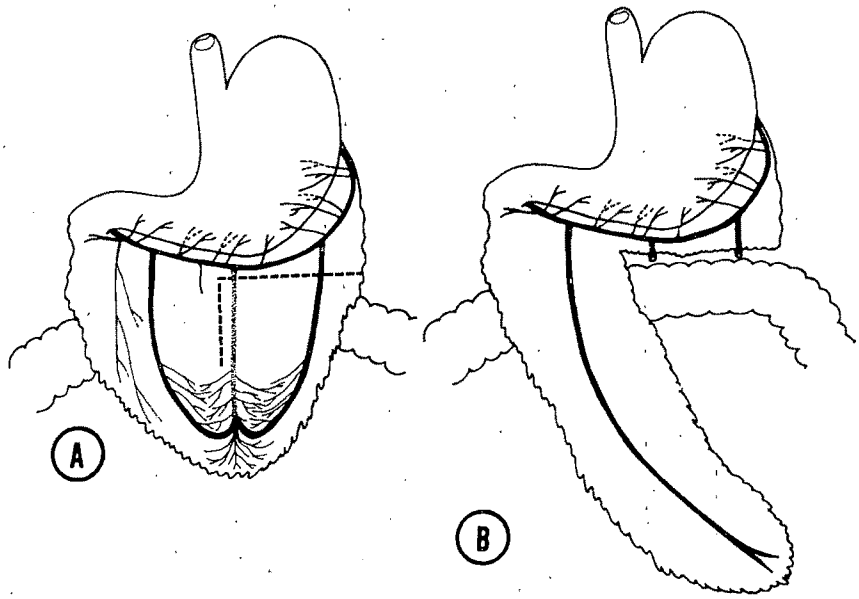


Fig. 5. *A*, Incision of omentum *(dotted lines)*. *B*, Omental pedicle is unfolded.

Table I. Clinical characteristics

	Original disease			*New disease*			
Patient	*Type*	*Cervical stage*	*Cancer date*	*Age (yr)*	*Type*	*Stage*	*Date*
A. H.	Squamous	IB	1965	46	Endometrial adenocarcinoma	I	1981
M. S.	Unknown	Unknown	1955	56	Mixed mesodermal sarcoma, fibrotic pelvis	II	1982
J. S.	Squamous	IB	1981	33	Adenocarcinoma of cervix	IB, occult	1982
T. H.	Squamous	IIB	1982	35	Recurrent hemorrhage, dysplastic endometrium		1983
E. H.	Squamous	IB	1971	45	Endometrial adenocarcinoma	IV	1985

Table II. Surgery, hospitalization, and outcome

	Surgery		*Type of bladder catheter*	*Length of bladder drainage (days)*	*Hospitalization*		*Outcome*	
Patient	*Operating time*	*Omental graft*			*Duration (days)*	*Complications*	*Fistula*	*Status*
A. H.	2 hr 30 min	Pedicle	Suprapubic	5	7	None	None	No evidence of disease
M. S.	4 hr	Pedicle	Urethral	1	20	Postoperative ileus, tumor at margin	None	Alive with disease
J. S.	2 hr 50 min	Pedicle	Suprapubic	12	7	None	None	No evidence of disease
T. H.	3 hr 45 min	Pedicle	Suprapubic	40	7	None	None	No evidence of disease
E. M.	2 hr 50 min	Direct attachment	Suprapubic	11	9	Inadequate voiding in hospital	None	Alive with disease

that its most dependent portion could be sutured to the vaginal apex without tension on it. The other patients had to have omental lengthening so that the omentum could be attached to the vaginal apex.

Omental lengthening by formation of an omental pedicle graft is based on the vascular anatomy of the omentum.[4, 5] The omental blood supply arises from the right and left gastroepiploic arteries, respectively (Fig. 1). The right and left omental arteries provide the main blood supply to the omentum. The right and left omental arteries anastomose with one another through the distal omentum (Fig. 2).

When the omentum appears to be long enough so that a partial transection of the omentum below the transverse colon would give adequate length to reach the vaginal apex, a reversed J-shaped partial transection of the omentum may be performed with the use of absorbable sutures (Fig. 3, *A*). The freed portion may then be rotated downward to be fixed to the vaginal apex with absorbable sutures (Fig. 3, *B*). The right omental artery is usually the largest omental artery. It is preserved by transecting the left side of the omentum and the left omental artery.

When the omentum is short, it must be dissected free from the transverse colon to obtain additional length for the pedicle. The undersurface of the omentum is attached to the anterior surface of the transverse colon by peritoneal adhesions and small blood vessels. This attachment is visualized by lifting up the distal omentum. Adhesions may then be transected and blood vessels ligated (Fig. 4).

The undersurface of the omentum has the greatest degree of freedom from the mesentery of the transverse colon on the left side. It may adhere to the transverse mesocolon on the right side. Dissection of the undersurface of the omentum from the left side usually avoids the need to dissect the omentum from the transverse mesocolon.

The incision down the middle of the omentum should be low enough to unfold the pedicle (Fig. 5). The new distal end of the pedicle is then attached to the vaginal apex by two or three interrupted, absorbable sutures. The pedicle is not sutured to the bladder or to the pelvic sidewall. The pelvic peritoneum is closed from each lateral sidewall to the lateral edge of the omental graft when possible.

Results

The total operating time for each of these patients was long and reflects the time spent carefully performing the hysterectomy. The omental graft adds less than 15 minutes to the procedure.

Four of the five patients had no significant postoperative problems. Patient E. M. delayed hospital discharge an additional 2 days in an attempt to achieve normal voiding and catheter removal. Only Patient M. S. had a complicated recovery. This was due to slow resumption of peristalsis.

When possible, suprapubic catheterization of the bladder was performed to allow prolonged bladder drainage if necessary and to prevent bladder trigone injury. Since the space of Retzius of Patient M. S. was obliterated by fibrosis, transurethral bladder drainage was performed. Patients J. S. and T. H. had arbitrarily long periods of catheterization. Patients A. H., E. M., and M. S. had catheters removed when they were able to void satisfactorily.

Two patients developed their abnormalities 1 year after radiation therapy. Three developed abnormalities late, 14 to 30 years after treatment. Although all patients were thought to have clinically localized disease, two had more advanced disease, found at the time of operation.

Despite the above factors, none of these patients developed any postoperative fistulas. Urinary, bowel, and vaginal tissues have remained intact. These organs have remained as functional as they were before operation was performed.

Comment

Vesicovaginal fistulas after complete pelvic radiotherapy and hysterectomy occur most commonly 5 to 12 weeks postoperatively.[2] This time delay suggests that the fistula may occur from progressive ischemia of the bladder base. The omentum has been used in several anatomic areas for improving tissue vascularity.[4] Improved vascularity to the bladder base and vaginal apex may be the reason that the omental graft may prevent fistula formation.

The length of bladder catheterization of patients in this series was variable. The absolute length of time of catheterization is probably not important. Currently suprapubic catheters are being removed when the patient is able to void without retaining significant amounts of residual urine.

The omentum is often too short to reach the vaginal apex without undue traction. The use of omental lengthening techniques has allowed easy application of the intact omentum to the vaginal apex and bladder base.

Abdominal hysterectomy with omental graft has resulted in no fistulas to date in this series. This outcome parallels the experience of Graham and Goplerud[2] and suggests that the omental pedicle graft may prevent fistula formation in patients undergoing abdominal hysterectomy who have previously received full radiation therapy for cervical cancer.

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Discussion

Dr. John J. Stapleton, Reno, Nevada. When Roentgen discovered the x ray 90 years ago, he inaugurated a great service to women with carcinoma of the cervix. Unfortunately, he also bestowed on the gynecologic surgeon who may subsequently operate on these patients a formidable task—adequate surgery without subsequent fistula formation. After the first successful radium implant by Cleaves, in New York City, Forssell established the Radiumhemmet in Stockholm. From this institution, Heymann and, subsequently, Kottmeier dictated the treatment of cervical carcinoma to the entire world for more than half a century. The morbidity and mortality rates for radical surgery initially reported from the clinics of Wertheim and Okabayashi were unacceptable to most gynecologists. It was not until Meigs' definitive work, "Surgical Treatment of Cancer of the Cervix," was published in 1954 that primary surgery for this disease was reborn. This work not only heralded an era of two choices of treatment, but in the effort to effect even better results, various associations of radiation and surgery subsequently emanated forth. Certain combinations resulted in a high incidence of postoperative fistula formation. Unfortunately the type of fistula was not usually amenable to the classic primary closures of Sims, Latzko, Boari-Casati, or Moir but necessitated diversionary surgery. Prevention, rather than repair, became the most important consideration.

I thank Dr. Petty and his colleagues for this excellent descriptive and informative presentation, which demonstrates the employment of omentopexy for prophylaxis against fistula formation when surgery and radiation are combined. Although Walters first described the utilization of the omentum to repair a vesicovaginal fistula in 1937, the major body of work reporting the use of this pedicle of vascular fat is found in the genitourinary literature; renal and ureteral wraps to prevent subsequent leaks are commonly employed. Indeed, until I reviewed the literature to discuss this article, I had no idea of the number and variety of uses for the omentum. My knowledge was limited to removal for malignancy or development of an omental carpet after exenteration.

I have several questions for Dr. Petty. Do you advocate prophylactic omentopexy at the time of primary surgery for those patients who will have or most likely will have postoperative irradiation? Would you recommend an omental graft for primary benign surgery when there is extensive denuding of the pelvic contents but no invasion of the gastrointestinal or genitourinary organs? Would you recommend this procedure even if a significant and noncosmetic extention of the initial incision is required?

I question the preference for suprapubic, as opposed to urethral, drainage. I strive to avoid any unnecessary invasion of the irradiated bladder. Finally, will you comment on pelvic drainage with respect to method and time? This alone may be responsible for the almost twofold increase in fistula formation between Symmonds' series and Graham and Goplerud's first group.

Dr. Petty (Closing). You asked about the prophylactic use of omentopexy in the patient who was to receive postoperative radiation therapy. I have not done that. It would be worthwhile if one were finding the occurrence of many fistulas from this treatment combination. There are many other uses of the omental pedicle graft. I have used it to cover a disrupted appendicele stump in one patient. In another, in whom the vaginal apex sloughed after removal of an infected postpartum uterus, the pedicle graft was an effective barrier for and allowed healing of the open, necrotic vaginal apex. The omental pedicle graft is useful for improving the success rate of repairing large vesicovaginal fistulas. These fistulas are approached abdominally and the pedicle graft is interposed between the repaired bladder and vaginal portions of the fistula. As in the fully irradiated cervix, I have used the pedicle graft in the patient with a partially irradiated cervix, if at the time of hysterectomy the bladder base is found to be thin or the bladder is injured. Finally, the omental pedicle has been used by me for its absorptive capacity. It can keep permanently resolved large abdominal peritoneal or retroperitoneal cysts. The cyst wall is opened, and the graft is inserted and sutured to the opening of the cyst. Other uses of this simple procedure may certainly be developed in gynecologic surgery.

Expanded toxemia syndrome or gestosis

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The expanded toxemia syndrome or gestosis refers to polysymptomatic diseases that are associated with pregnancy. This report discusses those cases without initial hypertension or proteinuria that were "cured" by delivery and were associated with maternal and fetal morbidity (usually intrauterine growth retardation). A list of suggested tests is presented to document gestosis in pregnant women with medical illnesses. Unlike preeclampsia, gestosis may occur at almost any time in pregnancy. (AM J OBSTET GYNECOL 1986;154:1227-33.)

Key words: Toxemia, expanded syndrome; gestosis

Since the late nineteenth century, it has been recognized that pregnant women may manifest disease of several organs, many times in association with hypertension. Together, the 1913 edition of DeLee's *The Principles and Practice of Obstetrics*[1] and the 1912 edition of *Williams' Obstetrics*[2] considered as examples of toxemia of pregnancy diseases such as headaches, hyperemesis, dermatitis (herpes gestationalis, impetigo herpetiformis), yellow atrophy of the liver, chorea gravidarum, psychosis, and hypertension. In 1937, the American Committee on Maternal Health presented a more confined classification of toxemia, which was limited to hypertensive disease, renal disease, preeclampsia (severe or mild), eclampsia, vomiting of pregnancy, acute yellow atrophy of the liver, and "unclassified" toxemia.[3] However, the fourteenth edition (1971) of *Williams' Obstetrics* spoke disparagingly of the term "toxemia" and further limited the disease to only hypertensive disorders.[4] While there has been continuous redefinition of the abnormality with each new edition of *Williams' Obstetrics*, the seventeenth (1985) edition mentions the term toxemia only to condemn it as "unsatisfactory."[5]

The editors of the latest edition of *Williams' Obstetrics* do not discuss how their suggested term for toxemia, pregnancy-induced hypertension, applies when a pregnant woman is desperately ill with pregnancy-related thrombocytopenia, subcapsular liver hematoma, and liver dysfunction but has little or no hypertension. While such cases of toxemia occur infrequently, they remain a significant cause of preventable maternal deaths,[6] in part, because such pregnancy-induced illnesses are often considered medical rather than obstetric in origin. Thus there appears to be a modern need for retaining the obstetric concept of toxemia.

Presented here are examples of polysymptomatic diseases (gestosis) involving more than two organ systems occurring in pregnant women, which are resolved by delivery. The term gestosis as used here refers to a pregnant woman who is ill and has symptoms arising from several target organs.

Material and methods

Fifty-nine normotensive ill pregnant women, seen either at the University Hospital in Denver or in Omaha, with possible gestosis (according to the definition of multiple target organs being affected), were studied for presence of elevated digoxin and human chorionic gonadotropin (hCG) levels, abnormal uterine souffle, venous reactivity, and relative hypovolemia. All women were apparently in good health without disease between pregnancies. There were no maternal or perinatal deaths.

Venous reactivity was obtained with the leg plethysmographic technique and plasma volume was determined with the Evans Blue method as previously described.[7] Venous reactivity was considered abnormal when the relative resistance ratio was >0.13 and plasma volume was abnormal when the value was below 48 ml/kg. hCG was considered abnormal when the urinary Gravidex test was positive after the urine had been diluted 1:20 with saline solution. The placental bed was located with ultrasound and the maternal flow was considered abnormal when Doppler examination showed reduced diastolic flow either on the cathode-ray tube screen (with a dicrotic notch) or with the audible monitor. The serum digoxin level was determined with the Abbott fluorescence polarization TDX system with serum values >0.2 ng/ml of digoxin equivalent being considered abnormal.

Results

Of the 59 initial subjects, in 12 patients either sustained hypertension developed or the medical problem

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Table IA. Abnormal tests with percentage of pregnancy-induced illness

	Plasma volume	*Urinary hCG*	*Serum digoxin*	*Uterine souffle*	*Venous reactivity*
No. of subjects tested	29	31	27	32	37
Tests abnormal (%)	69	42	67	63	78
Pregnancy-induced illness with abnormal test (%)	62	85	71	63	38

Table IB. Examples of pregnant women with apparent gestosis from 23 to 35 weeks gestation

Dominant disorder	*Previous pregnancy*	*Maternal plasma volume (ml/kg)*	*Newborn weight percentile*	*Abnormal laboratory findings**	*Subsequent pregnancy*
Toxemia Skin rash	Toxemia Skin rash	44	11th	Oligohydramnios on ultrasound, IUGR, skin biopsy showing macular rash	Repeat toxemia, rash
Positive antinuclear antibodies	Normal	32	8th	Tests "consistent" with lupus	Apparent lupus
Cortical blindness	NA	38	4th	Bun 24 mg/100 ml SGOT 243 units	
Thrombocytopenia	Thrombocytopenia	27	12th†	Platelets 18,000/mm³ SGOT 290 units Amylase 460 units BUN 18 mg/100 ml	
Nephrotic syndrome	Normal	36	9th	Serum cholesterol 680 BUN 24 mg/100 ml SGOT 360 units	Nephrotic syndrome
Liver dysfunction	Normal	38	11th	Serum amylase 190 units SGOT 190 units Platelets 56,000/mm³ Subcapsular liver hematoma on ultrasound	Liver dysfunction
Pancreatitis	Normal	44	5th	Serum amylase 850 units SGOT 110 units Platelets 52,000/mm³	"Pancreatitis"
Cholelithiasis	NA	46	10th	Gallbladder wall thickening and cholelithiasis on ultrasound	

See text for details. IUGR = Intrauterine growth retardation; NA = not available; BUN = blood urea nitrogen; SGOT = serum glutamic-oxaloacetic transaminase.

*Current pregnancy.

†Lowest weight.

resolved before delivery. In addition, 10 demonstrated signs of normal plasma volume expansion (decline in hematocrit, normal amniotic fluid volume, and a loud pulmonic flow murmur). Thus 37 subjects were available for laboratory confirmation with our special tests for gestosis. Plasma volume determinations were performed in 29 patients (78%), urinary hCG levels were estimated in 31 (83%), serum digoxin levels were determined in 27 (71%), placental souffle diastolic flow was monitored in 32 (84%), and venous reactivity was examined in 37 (100%) cases. Table IA shows the percentages of the abnormal special tests that were apparently associated with pregnancy-induced illness. A pregnancy-induced illness was defined as one that persisted through delivery but was apparently resolved within a 2-week postpartum period.

There were 10 cases of pregnancy-related medical problems (albuminuria, blindness, dermatitis, pruritis, headaches, persistent heart burn, or upper abdominal pain) in which all patients had abnormal hCG levels, digoxin levels, venous tone, plasma volume, and uterine souffle. In Table IB are presented eight examples of gestosis with an initial blood pressure <140/90 torr and no evidence of diabetes mellitus at hospitalization. In addition to having multiple target organs affected, the fetuses were growth retarded. There were four other women who also apparently had pregnancy-induced illness, but one had no other abnormal tests and two had abnormal venous tone and were hypovolemic. One fetus was growth retarded.

Comment

As shown in Table II, a case of gestosis is a "sick" pregnancy with increased risk of perinatal and maternal morbidity and/or mortality. Hypertension may be severe but is often mild or absent. While gestosis is usually demonstrated in the third trimester of the first pregnancy, it may occur during any stage of any preg-

Table II. Expanded toxemia syndrome or gestosis

1. Variation in target organ expression described for more than 100 years[10]
2. Frequency of various target involvement apparently influenced by race. The two apparent extremes are in blacks, who often have only preeclampsia, and in Native Americans, who frequently have the polysymptomatic forms
3. Usually associated with failure of uterine spiral arteries to dilate and plasma volume expansion to occur (hypovolemia and hemoconcentration)
4. Often associated with elevated maternal hCG or "hyperplacentosis"
5. Fetal growth retardation or dysmaturity usually present. Oligohydramnios may occur for only short time periods during pregnancy
6. Gestosis can be threatening maternal and fetal disease, even in absence of hypertension
7. Maternal illness resolved by termination of pregnancy

Table III. Target organ or disease complexes of expanded toxemia syndrome

1. Autoimmune: negative or positive antinuclear antibodies; positive platelet antibodies (with thrombocytopenia); lupus-like syndrome
2. Central nervous system: seizures; headaches; cortical blindness
3. Dermatitis: herpes gestationalis (pemphigoid gestationis); Spangler's papular dermatitis; impetigo herpetiformis; prurigo gestationis (polymorphic eruption of pregnancy; toxemia rash of pregnancy
4. Eyes: retinopathy; retinal detachment
5. Hypertension: gestational hypertension; chronic hypertension
6. Hypovolemia: hemoconcentration; prerenal azotemia; hyperviscosity
7. Gallbladder: thickened wall; incidental cholelithiasis
8. Gastrointestinal: duodenal ulcer; gastrointestinal bleeding; upper abdominal pain responding to cimetidine
9. Liver disease: hyperemesis gravidarum; intrahepatic cholestasis; gestosis (toxemia); acute fatty liver; hemolytic anemia; megaloblastic anemia; subcapsular liver hematoma
10. Pancreatitis: elevated serum amylase, frequent in Native Americans
11. Preeclampsia: pregnancy-induced hypertension; edema-proteinuria-hypertension gestosis
12. Thrombocytopenia: idiopathic thrombocytopenia purpura; thrombotic thrombocytopenic purpura; hemolytic uremic syndrome; gestosis
13. Renal: nephrotic syndrome; preeclampsia; acute renal failure

nancy, even if the previous pregnancies were apparently normal. Preeclampsia or gestational hypertension, while often a very significant part of the gestosis complex, will not be discussed since the therapy is well known. Instead, the expanded toxemia syndrome will be presented from the concept of "gestosis," the "toxemic" dermatitis, autoimmune diseases, spiral artery and plasma volume changes, hyperplacentosis, venous reactivity, and technique of diagnosis.

Gestosis. Some of the clinical features of gestosis are presented in Tables II and III. In 1975, I presented to this society a series of cases of pregnant women with multiple organ involvement including liver abnormalities, gastrointestinal bleeding, blindness, etc. The patients were selected because they all had pregnancy-related thrombocytopenia and elevated serum transaminase values. I termed their disease process severe preeclampsia, although some of the pregnant women were not very hypertensive, had normal urinalysis results, and were in no apparent danger of having seizures. Nevertheless, they all were ill. In addition, many of these women were multigravid, some having had apparently normal first pregnancies. Since Chesley[8] has, at least for study purposes, strictly defined preeclampsia as limited to the first pregnancy and to pregnant women with abundant albuminuria, another term is needed to describe this expanded toxemia syndrome.

After World War II, the German-speaking obstetricians introduced the term gestosis. They used the letters EPH to refer to edema, proteinuria, and hypertension (EPH gestosis) and other letters to refer to symptoms such as thrombocytopenia (T) or liver disease (L).[9] In a second series of cases, we attempted to apply this terminology to describe the expanded toxemia syndrome as "EPH gestosis, type B," with type A being only those with severe preeclampsia.[10] This awkward term was replaced in the literature by Weinstein[11] with the acronym "HELLP syndrome." His term was well accepted, yet it often was applied to a pregnant patient with a subacute condition (in no immediate need of "hellp") who usually did not have clinical hemolysis, may not have had hypertension, and may have had involvement of organs other than those expressed in the term.[11] Since the term "toxemia" has been expunged from American textbooks and medical journals, it would appear that "gestosis" is the next best simple term to describe the expanded toxemia syndrome. Two conditions that are not usually considered examples of toxemia or gestosis, pregnancy-related dermatitis and autoimmune disorders, are also discussed.

Dermatitis. Papular dermatitis of pregnancy as described by Spangler et al.[13] is associated with elevated maternal hCG levels, depressed estriol levels, and a high fetal mortality rate. Other pregnancy-related dermatitides have been considered as toxemic rashes of pregnancy because of their associated maternal and fetal problems.[14] We described a series of cases of pruritus of pregnancy associated with elevated maternal hCG levels and maternal and fetal complications.[15]

Autoimmune disease. Autoimmune diseases during pregnancy are difficult to classify. Farnam et al.[16] concluded that the incidence of positive antinuclear anti-

Table IV. Laboratory tests to document presence of gestosis*

Test	*Target organ or system*
Antinuclear antibodies	Autoimmune disease
Amylase, serum	Pancreas
hCG[15]	Hyperplacentosis
Creatinine/blood urea nitrogen (serum)	Kidney, hypovolemia
Digoxin, serum	Natriuretic hormone
Glucose, blood	Hypoglycemia (liver)
Hematocrit	Hemoconcentration
Iron, serum	Erythropoiesis
Partial thromboplastin time, activated	Lupus anticoagulant Cardiolipin antibodies
Platelet antibodies	Thrombocytopenia
Platelet count	Thrombocytopenia
Pulmonary systolic murmur	Hypovolemia
Transaminases, serum (aminotransferase)	Liver
Ultrasound (level II)	Subcapsular liver hematoma Thickened gallbladder Oligohydramnios Dysmaturity
Uterine souffle with Doppler for diastolic flow	Presence of uterine arteriovenous fistulas

*Two or more tests should be positive in addition to chief complaint for diagnosis of gestosis.

bodies in pregnant women is significantly higher than in nonpregnant women. Depending on the laboratory techniques, from 5% to 100% of pregnant women with gestosis-associated thrombocytopenia have platelet antibodies. I have examined four patients with a systemic lupus erythematosus–like syndrome during pregnancy, which cleared with delivery, only to recur with subsequent pregnancies. Pregnant patients with apparent autoimmune disease often respond to steroids.[12]

Spiral artery changes and plasma volume expansion. In 1967, Brosens et al. demonstrated that structural alterations occur in the human uterine spiral arteries found in the decidual part of the placental bed. As a consequence of the action of cytotrophoblast on the spiral artery vessel wall, the normal musculoelastic tissue is replaced by a mixture of fibrinoid and fibrous tissue. The small spiral arteries become converted to large tortuous channels creating low-resistance channels or arteriovenous shunts. As early as 1903, Arguad, after studying the uterus in various pregnant mammals, noted these same spiral artery changes. He claimed these spiral artery alterations were encountered only in mammals adapted to live in the upright position during pregnancy. Brosens termed these normal spiral artery alterations "physiologic changes."

In dogs, when a surgically prepared arteriovenous shunt is opened, there soon appears a marked increase in the plasma volume, cardiac output, and retention of sodium of the animal. An apparent anemia occurs as the red blood cell mass is slower to expand. The reverse happens when the shunt is closed. The situation of opening arteriovenous shunts then is very much like early normal pregnancy, where there is an early increase in plasma volume and a resulting physiologic anemia as the red blood cell mass is slower to expand. Immediately after delivery, with closure of the placental shunt, associated diuresis and natriuresis occur. When the spiral arteries fail to undergo these "physiologic changes," as defined by Brosen et al., fetal growth retardation often occurs with preeclampsia. In 1983, Campbell et al. used gated, pulsed Doppler ultrasound to study the uterine arcuate arteries serving the spiral arteries and placenta in pregnant women. Among the patients who showed evidence of failure of the spiral arteries to dilate and increased vascular resistance, there was subsequently a high frequency of proteinuric hypertension, poor fetal growth, and fetal hypoxia.

Wells et al. demonstrated, in 1984, that the decidual spiral arteries that have been attacked by the cytotrophoblast have a fibrinod matrix that develops amniotic antigens, apparently to maintain the structural integrity of the vessel wall. Thus there are histologic and immunologic explanations for the presence or absence of the marked increase in uterine blood flow and plasma volume expansion seen in human pregnancies.

Hyperplacentosis. The concept of hyperplacentosis presented by Scott, in 1958, refers to an increased placental activity, usually increased maternal levels of hCG. Jeffcoate and Scott later demonstrated that many preeclamptic women have elevated hCG levels or hyperplacentosis, and subsequent studies have indicated that severe preeclampsia is associated with elevated maternal serum and urine hCG levels. Pregnancy-related dermatitis, especially pruritus associated with poor fetal outcome, is also associated with increased hCG levels.

Venous reactivity. In 1937, Nicholson J. Eastman suggested that instead of the concept of the renal system or the so-called "low reserve kidney," the dominant abnormality in toxemia of late pregnancy is the vascular system. The basis of his argument was "the constancy at which spasm of the retinal arterioles is met in these disorders." By contrast, our own studies have suggested that it is the venous rather than the arteriolar activity that is the significant abnormality in women with preeclampsia, as the veins contain at least 75% of the blood volume of pregnant women. However, whenever the sympathetic nervous system increases the vasomotor tone of the veins, it also constricts the corresponding arterioles. While venous vasodilatation may be incidentally achieved with therapy intended to produce arteriolar dilatation, our studies suggest that the apparent abnormal venous circulation should receive direct attention in the therapy of gestosis.[7]

Diagnosis. In order to appreciate the frequency of gestosis, it is necessary to perform several tests routinely in all pregnant women having significant medical abnormalities. Table IV lists some of the laboratory tests

reported to be abnormal in the expanded toxemia syndrome or gestoses. Unfortunately, the incidence of elevated serum transaminase levels in apparently normal pregnant women of the various racial groups, at term, is not known. Because of the increased platelet aggregation with gestosis, apparent thrombocytopenia determined on an electrical counter should be confirmed either by peripheral blood smear or by manual platelet count. To confirm normal plasma volume expansion, we auscultate with a stethoscope for the presence of a loud maternal pulmonic systolic flow murmur, and for uterine spiral artery dilatation we use a continuous Doppler device for the presence of loud diastolic flow in the maternal placental bed (iMEX 301).

Although we have a 9-year experience with the Swan-Ganz catheter technique, I no longer use it in gestosis cases.[10] While cardiac outputs and pulmonary capillary wedge pressures may be increased, unchanged, or decreased, these determinations have not been of clinical benefit and the procedure has been associated with significant maternal morbidity. By contrast, estimation of maternal venous tone is without apparent risk and in my experience provides clinically useful information.[7]

Therapy. With the exception of acute treatment for preeclampsia, obstetricians disagree on the concepts of treatment of gestosis. My concept is that the failure to dilate spiral arteries and to adequately expand plasma volume in the pregnant woman evokes increased venous reactivity and multiple maternal organ dysfunction. While the absence of the "physiologic changes" of the spiral arteries probably cannot be corrected, appropriate therapy can modify the abnormal venous reactivity and its associated maternal hypovolemia, if the diagnosis is made before the development of serious illness. Drugs that decrease venous resistance and lower venous tone include amyl nitrite, furosemide, nitroglycerin, prazosin, and tranquilizers such as hydroxyzine. If standard therapeutic measures do not correct the hypertensive and hypovolemic states by reducing blood pressure and venous reactivity, then treatment with prazosin and simultaneous expansion of plasma volume with 5% or 25% albumin seems appropriate. Steroids (topical triamcinolone acetonide, prednisolone tablets, or intramuscular dexamethasone) have been reported to improve the abnormal findings of patients with gestosis (dermatitis, liver dysfunction, platelet antibodies, lupus-like symptoms, and/or thrombocytopenia). Cimetidine often improves symptoms in those with upper abdominal pain and/or elevated amylase levels. Heparin is not indicated, but platelet transfusions are often successful in the short-term treatment of thrombocytopenia.

The use of diuretics in preeclampsia has been condemned by obstetricians on the basis of the concept that they reduce blood volume or placental perfusion.[5] However, as noted in a review of randomized trials of diuretics in preeclamptic pregnancy by Collins et al., diuretics apparently reduced the incidence of stillbirths by one third. Many diuretic agents decrease venous tone and increase venous capacitance and their hypovolemic effect is temporary. While such agents are often used to prevent pulmonary edema during the course of active plasma volume expansion with colloid agents in preeclamptic patients, part of their efficacy may be due to their effects as venous vasodilators.

In conclusion, it serves no useful purpose to categorize all medical diseases associated with pregnancy as "gestosis"; rather only those associated with detrimental maternal and/or fetal effects should be so classified. This article has emphasized the expanded nature of the syndrome, but accepted concepts and therapy[5] for preeclampsia-eclampsia must not be ignored.

The additional clinical implications of the concepts of "gestosis" beyond those of simple preeclampsia or pregnancy-induced hypertension are: (1) there are several mild to severe polysymptomatic diseases with or without multiple target organ involvement associated with pregnancy, which regress with delivery; (2) the common feature in gestosis of relative maternal hypovolemia indicates that many of these pregnancies are at risk for serious maternal and fetal complications, even in the absence of maternal hypertension; (3) while each one of the symptom complexes or target organs may have a suggested form of therapy, the presumed failure of the "physiologic change" of the spiral arteries or other uteroplacental abnormality probably limits any therapy other than delivery; (4) nevertheless, in addition to standard preeclampsia therapy, steroids and/or techniques to achieve plasma volume expansion appear to aid many of the patients with gestoses; (5) while platelet transfusions may be necessary, heparin should not be used; (6) the gestoses are usually most severe in (or even limited to) the third trimester of the first pregnancy, but the multigravid woman may be a victim of the severe multiple organ involvement even after a normal prior pregnancy; (7) after a pregnancy with gestosis, there is usually a reduced risk with subsequent pregnancies, but it may also be more severe or in a different form in subsequent pregnancies. For those patients having had pregnancy-related thrombocytopenia, prophylactic low-dose aspirin therapy may be indicated early in subsequent pregnancies. Maternal tranquility and adequate diet may also ameliorate the disease process.

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A full list of references will be furnished on request.

Discussion

DR. EDWARD J. QUILLIGAN, Sacramento, California. This article by Dr. Goodlin examines the concept that pregnancy can induce or be associated with abnormality in an organ or organs other than the vasculature or kidneys in a manner similar to pregnancy-induced hypertension. He calls this the expanded toxemia syndrome or gestosis. He examined 59 pregnant women who were in apparent good health between pregnancies and who developed multiple organ disease without hypertension during pregnancy. The examination included venous reactivity, blood volume, and hCG. Ten pregnant women had an elevated venous reactivity and hCG level if I read the article correctly. He gave eight examples of patients with gestosis including toxemia, skin rash, positive antinuclear antibody test, cortical blindness, thrombocytopenia, nephrotic syndrome, liver dysfunction, pancreatitis, and cholecystitis. I do not know what happened to the other two and my first question to Dr. Goodlin would be why he did not include them.

He went on to develop the hypothesis that gestosis is due fundamentally to a failure of adequate plasma volume expansion caused, at least in part, by a failure of adequate dilatation of the spiral arteries, the same concept that some feel is responsible for pregnancy-induced hypertension. He then discussed the diagnosis and therapy of gestosis in view of his concept of the pathogenesis. The diagnosis should feature reduced plasma volume, and therapy is directed toward increasing plasma volume. This is somewhat oversimplified since there are other tests involved in the diagnosis of thrombocytopenia, for example, and other therapies. These tests and therapies will vary depending on the organ system or systems involved.

Dr. Goodlin's concepts may be correct. He certainly has developed many novel, interesting, and important concepts during the past several years; however, I think this one needs more proof before therapy is based on it. Equating venous reactivity with decreased plasma volume may be a quantum leap that needs proving. My next question would be why he did not compare plasma volumes in the 10 patients with abnormal venous reactivity with those of 10 of the patients with a disease similar to gestosis but normal reactivity and those of 10 normal control pregnancies at matched gestational ages. If this could not be done, was there any other evidence of failed volume expansion in the gestosis patients, such as an elevated hematocrit?

The failure of the spiral arteries to expand is due to the lack of trophoblastic invasion, which proceeds throughout the first and second trimesters. Examination of the basalis may show this change or lack of the same. Were any biopsies done with this in mind?

The work of Campbell quoted in this article is exciting in that it shows a reduction in ultrasonically determined uterine blood flow, which precedes the onset of pregnancy-induced hypertension However, these studies are not universally accepted as indicative of uterine blood flow and, assuming they are correct, similar studies need to be performed in the gestosis patients before one can assume reduced blood flow in these patients.

Finally I would like to say a word about therapy. Dr. Goodlin has suggested, and I quote, "If standard therapeutic measures do not correct the hypertensive and hypovolemic states by reducing blood pressure and venous reactivity, then treatment with prazosin and simultaneous expansion of plasma volume with 5% or 25% albumin seems appropriate." In my opinion use of plasma volume expansion with or without vessel dilators requires some very carefully controlled studies before it can be recommended. There have been too many instances of congestive heart failure with plasma volume expansion used in patients with pregnancy-induced hypertension.

As always, I enjoyed Dr. Goodlin's article because, whether he is right or wrong, he always has interesting and provocative ideas.

DR. MERLE ROBBOY, Newport Beach, California. I would like to ask Dr. Goodlin if he feels that the hyperemesis gravidarum syndrome is part of his gestosis picture. What would he do with a patient who has this disease that extends into the second trimester?

DR. JULIAN PARER, San Francisco, California. Dr. Goodlin, what you appear to have done is take a group of patients with abnormalities and discard hypertension as one of the diagnostic features. It seems to me that if you do a whole host of laboratory tests on a group of totally normal women, you are bound to come up with abnormalities in some of them. My question is how do these people present? What are their symptoms and how do you pick up this disease?

Dr. Richard Paul, Los Angeles, California. In the management of these patients, I wonder what role activity or nonactivity plays. Of what benefit is the therapy of bed rest? Does that improve the condition and, if so, by what mechanism?

Dr. Kelvin Churches, Carlton, Australia. I would be interested if Dr. Goodlin would explain why he so summarily dismissed the use of heparin, as this is fairly routine treatment for severe preeclampsia at the Royal Victoria Women's Hospital in Melbourne.

Dr. Goodlin (Closing). I will answer Dr. Churches' question first. The use of heparin in these cases can be a disaster. In my experience in the last 20 years I have seen these women being admitted having had 80 to 90 units of blood and plasma after thrombocytopenia was treated with heparin. They have associated subcapsular liver hematomas. Maternal deaths are associated with the use of heparin.

I think that heparin should never be used under these circumstances. As I tried to indicate, if thrombocytopenia is a significant problem, platelet transfusion works. I have had anesthesiologists and hematologists tell me that "platelet transfusion won't work because the associated antibodies will destroy the platelet," and, in my experience, that is not true. Bed rest, Dr. Paul, I agree is the *sine qua non* of all these therapies. I think the most successful way that one can achieve plasma volume expansion in hypovolemic pregnant women is with bed rest and tranquility.

Dr. Parer asked what symptoms these pregnant women had. I studied hospitalized women who had any symptoms of the various target organs that I mentioned who were pregnant. I do not know the percentage of otherwise normal pregnant women who have thrombocytopenia, but I assume that if they do have thrombocytopenia then that is significant. And the same with elevated liver enzymes. The digoxin levels have proved very useful for diagnosis. So I was simply looking at ill pregnant women who were hospitalized, who had all types of alleged medical diseases, and seeing if I could fit them into the definitions of gestosis. And, as I said, of the 59 that I studied, at least 10 of them had the "disease." To answer Dr. Quilligan, two of the women had a nephrotic syndrome and two had the lupus-like syndrome.

The issue of plasma volume expansion is for me an almost emotional one. I have become less enthused for the technique because nowadays we have to get women only to the twenty-eighth week of pregnancy in order for the neonatalogist to have an excellent outcome of the newborn infant. When I started this oncotic therapy in the 1960s we had to prolong pregnancy to 34 weeks. So plasma volume expansion, I will concede, really does not have the role I thought it had 15 or 20 years ago. I still think it is very useful for the signs of fetal distress in hypovolemic women or if you wish to use an epidural anesthetic. I think the group from Australia has shown in a very elegant way that plasma volume expansion works in women with toxemia or gestosis. However, it may not be necessary; it may be simpler just to deliver these women and let the neonatologist struggle with the neonate. Minipress (prazosin) is the only antihypertensive drug that appears to dilate veins. It is an α-adrenergic receptor blocker, and that is why I think it makes sense to use in expanding plasma volume in hypertensive pregnant women.

I think, at least from published results, I have seen more cases of pulmonary edema than anyone. In my experience it is easily handled with furosemide. Many physicians do not make the diagnosis of pulmonary edema because they do not obtain a chest x-ray film when dyspnea or chest pain occurs. The simplest way to avoid pulmonary edema, when using oncotic therapy, is to note whether the women undergo diuresis after the plasma volume expansion, or if there is a lowering of the hematocrit. If not, it is the wrong therapy. Another feature is to make certain that these women are sitting upright.

I think the uterine Doppler studies are well established, and I have used them since 1974. I attended the World Congress on Hypertension in Amsterdam last year and the United States was about the only country that did not have a paper on the use of Doppler and the arcuate artery or placental bed studies. The Soviet Union, Israel, Germany, Japan, France, England—all over the world people have picked up on this, and, to me, it is another example how America lags behind the rest of the world. The Doppler studies indicate by 22 weeks which pregnancies are at risk.

The group in London obtained a 2 cm cube of the placental bed in order to study the spiral arteries. They apparently invert the uterus at the time of cesarean section and obtain this tissue. I have never been able to obtain permission from our experimental study group for this project. We have tried repeatedly to study the spiral arteries with simple scraping or looking at the placental surface and have failed. I agree that the hematocrit is useful.

Liley, the great New Zealand investigator, showed, in 1970, that if a pregnant woman has a high hematocrit the odds are 4 to 5 that she is hypovolemic, and if she has a low hematocrit, she is not anemic four out of five times, but it is probable that the plasma volume has expanded.

As far as the study group is concerned, all of these women had plasma volume studies. As I recall, 40% were considered to be hypovolemic but did not have the required elevated hCG and digoxin levels or had normal Doppler studies. R values were high.

Severe hyperemesis can be gestosis and can be treated as toxemic. I have used steroids when the liver enzymes were elevated and oncotic therapy when there was hypovolemia after ruling out hyperthyroidism.

Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis

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In 110 midtrimester amniocenteses of the 3349 procedures done at the University of California, Davis, Medical Center between 1979 and 1984, the fluid obtained was noted to be discolored. The significance of this finding was evaluated with review of pregnancy history and outcome and by using biochemical techniques. A significant increase in miscarriage was seen in the pregnancies where discolored fluid was obtained (9%), in comparison with the entire group of pregnancies in which amniocentesis was done at the same institution over the same period (1.6%). This increase was most marked in the pregnancies in which, in addition to discolored fluid, there was a prior history of pregnancy bleeding. Thirty-four discolored fluid samples and 18 clear fluid samples were studied with spectrophotometry, electrophoresis, isoelectric focusing, and chromatography. These studies indicate that in most cases the discoloring pigment is blood rather than fetal bowel contents, as had been suggested by others. (AM J OBSTET GYNECOL 1986;154: 1234-40.)

Key words: Amniocentesis, midtrimester, amniotic fluid, discolored, abortion

Green or brown discoloration of fluid obtained at midtrimester amniocentesis has been a concern of many investigators. The incidence has been reported to vary between 1% and 7% of procedures performed. Association with a history of bleeding during early pregnancy and with prior unsuccessful amniocentesis has been noted. The significance of the finding has been a subject of much debate, with some authors noting fetal demise in as many as 30% of pregnancies with discolored fluid[1, 2] and others finding little or no increase in adverse pregnancy outcome.[3-7]

Surprisingly little investigation has been done into the cause of the discoloration. Various authors have assumed it to be blood,[6] either of fetal or maternal origin, or meconium.[1, 3-5, 7] Most authors base their statements about the cause of the discoloring substance on spectrophotometric studies,[3, 4] although it is clear that both blood and meconium give an absorbance peak in the 405 nm range.[8] Hankins et al.[6] have added a guaiac test (Hemoccult, Smith Kline & French Laboratories, Philadelphia) of the fluid. Allen[7] distinguishes between blood-stained fluid (brown) and meconium-stained fluid (green) on the basis of visual appearance, having excluded the brown fluid samples from his study.

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In this study an attempt was made to look at both the outcome of the pregnancy and the cause of the discoloration of the fluid.

Material and methods

For this study all amniocenteses in which the amniotic fluid was noted to be discolored by visual inspection by the obstetrician performing the procedure were selected from a series of 3349 amniocenteses performed for standard genetic indications. A total of 110 discolored fluid samples (3.3%) were evaluated. Charts were reviewed for pregnancy information routinely obtained prior to the procedure. Questions asked as part of the preamniocentesis counseling session included screening questions for pregnancy complications such as uterine cramping, bleeding, or illness, as well as questions on exposures to medications or x rays. The delivering physician was contacted for pregnancy outcome information, including date of delivery, length of gestation, birth weight, and any noted abnormalities of the infant. The incidence of adverse outcome was then compared with the outcomes seen in the entire amniocentesis group. In assigning pregnancies to abnormal outcome groups, each pregnancy was placed into only one such group. Thus, if a pregnancy ended with a premature live-born infant who subsequently died during the neonatal period, the pregnancy was assigned to the prematurity group and not to the neonatal death group. The decision to make this assignment was based on the fact that most of the information regarding outcome came from the obstetrician's office. Often those records did not include information about the ultimate

Table I. Comparison of pregnancy outcome in amniocenteses in which discolored fluid was obtained with all amniocenteses performed during the same time period*

Pregnancy outcomes	*Pregnancies with discolored fluids*		*All pregnancies*	
	No.	*%*	*No.*	*%*
Normal	82	74.5	2805	83.8
Miscarriages (<28 wk from LMP)	10	9.1	54	1.6
Lost to follow-up	7	6.4	237	7.1
Premature deliveries (<35 wk from LP)	6	5.5	53	1.6
Small for gestational age (<10%)	2	1.8	31	0.9
Stillbirths (≥28 wk from LMP)	1	0.9	22	0.7
Multiple gestations	1	0.9	44	1.3
Pregnancy electively terminated†	1	0.9	43	1.3
Undetectable abnormalities‡	0		48	1.4
Neonatal death	0		4	0.1
Abnormal pregnancies continued to term	0		8	0.2
Total	110		3349	

LMP = Last menstrual period.

*The amniocenteses were performed at the University of California, Davis Medical Center.

†In most elective terminations, a major fetal abnormality was documented prior to the decision to terminate.

‡These unanticipated abnormalities noted at delivery included cleft lip, congenital heart disease, syndactyly, etc. None would have been expected to be diagnosed by amniocentesis.

resolution of the neonatal course. Within the group of discolored fluid samples, outcomes of pregnancies in which there was a history of bleeding during pregnancy were compared with those in which there was no history of pregnancy bleeding.

Amniotic fluid samples from 52 procedures were examined by four independent analytic techniques: (1) sodium dodecyl sulfate (SDS) electrophoresis, (2) isoelectric focusing, (3) gel filtration chromatography, and (4) visible wavelength spectrophotometry. Of the fluid samples examined, 18 were chosen because they were judged to be clear by the clinician responsible for the amniotic taps and were considered to be representative of normal amniotic fluid. All of the remaining 34 fluid samples that were examined were from the group considered to be discolored, although the range of discoloration varied from slight to extreme. Gut contents from two fetuses electively aborted with prostaglandin (dinoprostone [Prostin E2], Upjohn Company, Kalamazoo, Michigan) at 15 and 18 weeks' gestation were subjected to the same analytic techniques as the amniotic fluid samples. The SDS electrophoresis followed the procedure of Fairbanks et al.[9] and employed resolving gels of 18% acrylamide. Gels of this unusually high acrylamide content were necessary to cleanly resolve the heme pigments and the pigments found in fetal gut contents. Isoelectric focusing was performed according to the methods of Vesterberg et al.[10] Gels of 7% acrylamide were used in most experiments. Gel permeation analysis of the amniotic fluids was carried out with the use of a G-100 Sephadex column (50 by 1.4 cm) with an elution buffer of 100 mmol sodium orthophosphate (pH 7.8).[11] Analysis of each chromatographic run was performed with a Gilson Holochrome column monitor measuring the A^{200} absorbance of the column effluent.[10] Spectrophotometric analysis was done with a Beckman DB photospectrometer by methods similar to those of Liley.[7]

Analysis of results. Table I compares the outcome of pregnancies in which discolored fluid was obtained to the outcome of the 3349 procedures done at the same institution during the same period. Most samples were obtained from procedures performed by one of us (F. W. H.). The overall outcome of most of these procedures was the subject of a previous report.[12] Ten women (9%) with discolored fluid went on to miscarry prior to 28 weeks' gestation, in comparison with 1.6% in the overall group. One pregnancy ended in stillbirth (1%). This did not differ from the overall stillbirth rate of 1%. Three fetuses had abnormal chromosome patterns. One fetus had trisomy 21 and was electively aborted. Two other fetuses had inherited balanced translocations. One female fetus had inherited a translocation (Y;22) from her father. She was examined at 1 year of age, and no abnormalities were noted. The other case involved a translocation (7;16) inherited from the mother. The pregnancy was complicated by fluid leakage and oligohydramnios prior to the amniocentesis. Delivery occurred prematurely, and the baby was transferred to another neonatal intensive care unit. After a difficult neonatal course, the baby was discharged home. Six women were delivered prior to 35

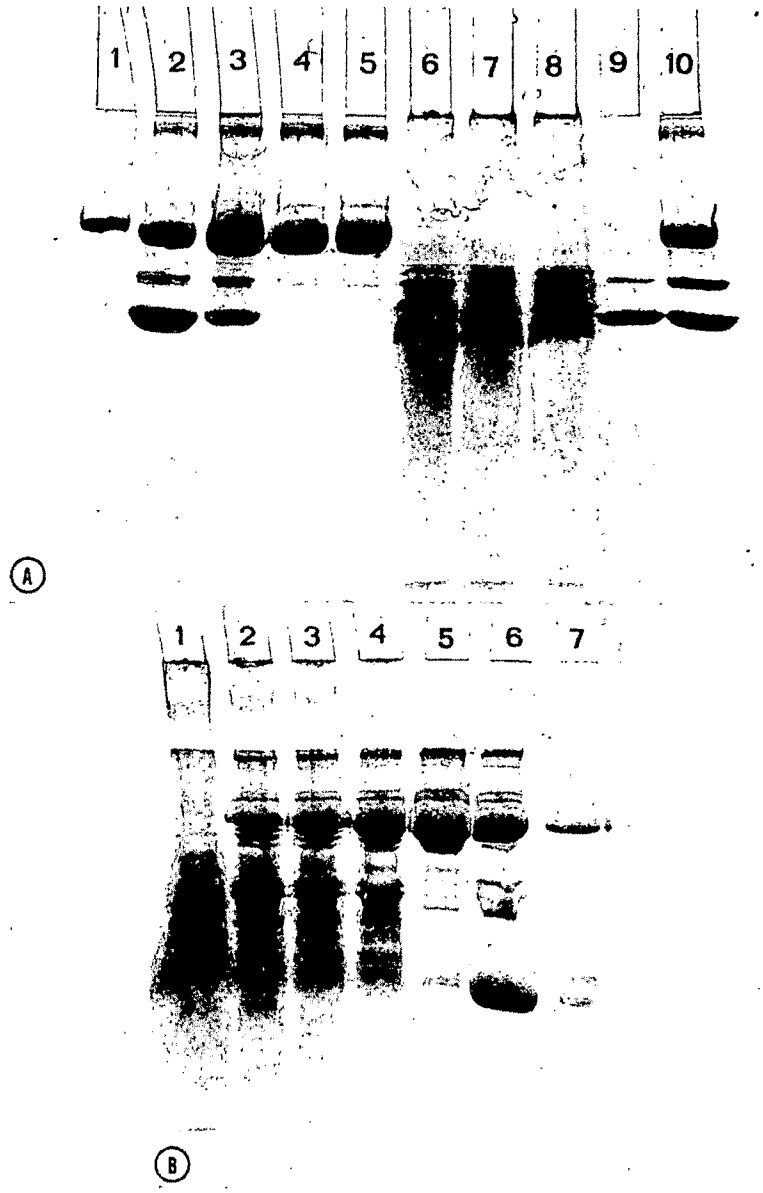


Fig. 1. *A*, SDS polyacrylamide gel (18%) of amniotic fluids: *lane 1*, 10 μg serum albumin; *lanes 2* and *3*, brown amniotic fluid; *lanes 4* and *5*, clear amniotic fluid (control); *lanes 6* and *7*, green amniotic fluid; *lane 8*, fetal gut contents (12 weeks); *lane 9*, 20 μg hemoglobin; and *lane 10*, 35 μg blood proteins from a 38-year-old man. Gels were stained with 0.1% Coomassie Blue R-250 in ethanol–acetic acid–H_2O (1:2:4). *B*, SDS polyacrylamide gel (15%) of green amniotic fluids. *Lanes 1* to *4* show decreasing quantities of green amniotic fluid (150 μl, 100 μl, 50 μl, and 25 μl) *lane 5 shows clear amniotic fluid, lane 6* shows brown amniotic fluid, and *lane 7* shows 10 μg serum albumin and 10 μg hemoglobin.

weeks' gestation, and two pregnancies produced babies that were small for dates. Of 44 sets of multiple gestation in which amniocentesis was done, discolored fluid was found in one. Discolored fluid was present in only one sac. These twins were dizygotic, and the twin in whose sac the discolored fluid was found was anencephalic. The pregnancy was continued to term, and both twins were live born. The anencephalic twin died at 5 days of age. The outcome group labeled "undetectable abnormalities" includes such birth defects as cleft lip and palate, congenital heart disease, and syndactyly. None of these abnormalities would have been expected to have been identified at amniocentesis or with the accompanying ultrasound test. In one procedure yielding clear amniotic fluid, the fetus was born at term with an abdominal perforation thought to be related to the amniocentesis. This was the only fetal injury seen in 3349 amniocenteses.

Of 110 pregnant women with discolored fluid samples, 69 (62%) gave a history of bleeding prior to amniocentesis (Table II). There were nine instances in which the discolored fluid was found on a repeat tap. In one case a repeat amniocentesis was done because of failure of cell culture from the first tap. The initial fluid was clear, but at the subsequent tap 3 weeks later, discolored fluid was obtained. In the remaining eight cases, in which repeat amniocentesis was done at a subsequent visit, no fluid had been obtained at the initial procedure. In all but two of these, both taps were done at the University of California, Davis, Medical Center. Two patients were referred when the initial attempt in the referring physician's office was unsuccessful. In three of these repeat taps, there was a history of bleeding in the pregnancy prior to the amniocentesis, and in all cases the pregnancy ended with the birth at term of a normal child. In nine of the ten miscarriages that occurred in the group with discolored amniotic fluid, a prior bleeding history was noted (13% of all pregnancies in which discolored fluid and a history of bleeding prior to amniocentesis were noted), in comparison with one (2%) in the group with discolored fluid but no history of bleeding. In addition, the twins, the small-for-dates babies, and three of the four premature infants were in the group with a preamniocentesis history of bleeding.

All discolored fluid samples demonstrated a spectrophotometric peak at 405 nm. High peaks were noted when the fluids were more visibly discolored. Although no peak was defined in the clear fluid samples, there was some absorbance at 405 nm in these fluids also. Aside from the height of the peak, no difference was noted among the variously discolored fluid samples on photospectrometry. On SDS electrophoresis, isoelectric focusing, and gel chromatography, it was possible to detect three different patterns. Of the 34 fluid samples, 31 contained a pigment that migrated with the blood of an adult man and was presumed to represent hemoglobin and its breakdown products. One fluid sample contained no blood pigments but contained a pigment that migrated with a pigment found in fetal gut contents. Two fluid samples contained both the heme pigment and the meconium pigment. Figs. 1 and 2 are examples of SDS electrophoresis and gel permeation chromatography, respectively. In an examination of lanes 6 and 7 of Fig. 1, *A*, it will be noted that the protein bands corresponding to serum albumin and other unidentified amniotic fluid proteins are absent. The bands are of course expected, and their absence is disconcerting. The difficulty, however, can be resolved by inspection of Fig. 1, *B*. In this illustration you see decreasing concentrations of green fluid, which shows increasing clarity of the expected protein bands. Apparently the materials from the green fluid are deleterious to the resolving efficiency of the Fairbanks system. At concentrations high enough to clearly show the green pigment, they completely interfere with the resolution of albumin, etc. Decreasing the concentration of the fetal gut contents did not change its staining pattern appreciably. Fig. 2 depicts G-100 chromotography of green fluid *(A)*, brown fluid *(B)*, and clear fluid *(C)*. The point of interest is the large peak at fraction 39 in Fig. 1, *A*, which is absent in *B* and *C*. This peak represents the low-molecular-weight green pigment seen in Fig. 1, *A* and *B*.

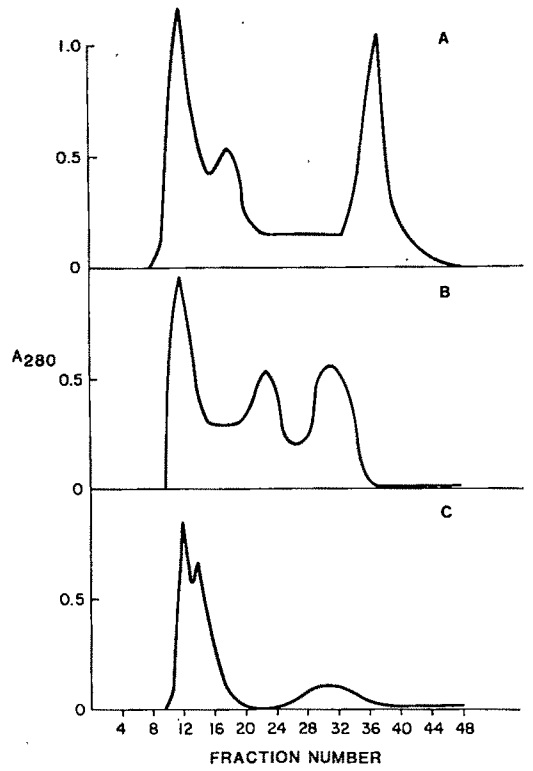


Fig. 2. Gel permeation chromatography of amniotic fluids. One-milliliter samples of green *(A)*, brown *(B)*, and clear *(C)* amniotic fluid was chromatographed on a 50 × 14 cm G-100 Sephadex column, with 100 mmol sodium orthophosphate (pH 7.8) used as the elution buffer. Fraction volume equaled 1.7 ml.

Comment

In this study of 110 discolored amniotic fluid samples, there was a 9% incidence of miscarriage, a significant increase over the 1.6% miscarriage rate of the total amniocentesis group ($p = 0.005$). We therefore conclude that these pregnancies are at increased risk for adverse outcome and that it is appropriate for the obstetrician who is managing such a pregnancy to note the finding. In particular, those pregnancies with both discolored fluid and a history of early bleeding are at highest risk, with a 13% miscarriage rate. Other adverse

Table II. Comparison of outcome of pregnancies with discolored amniotic fluid by presence or absence of history of bleeding in the pregnancy prior to the amniocentesis

Pregnancy outcomes	*Positive bleeding history*		*Negative bleeding history*		*Total*
	No.	*%*	*No.*	*%*	
Normal	47	68.2	35	84.2	82
Miscarriages (<28 wk from LMP)	9	13.0	1	2.4	10
Premature deliveries (<35 wk from LMP)	5	7.3	1	2.4	6
Lost to follow-up	3	4.4	4	10.0	7
Small for gestational age (<10%)	2	2.9	0		2
Abnormal pregnancy pregnancy terminated	1	1.4	0		1
Stillbirth (≥28 wk from LMP)	1	1.4	0		1
Multiple gestation	1	1.4	0		1
Total	69		41		110

LMP = Last menstrual period.

outcomes, such as stillbirth, chromosome abnormality, or other birth defects, did not appear to be increased in this group.

Although it is certain that the large majority (33 of 34) of discolored fluid samples examined by our group contained hemoglobin as the major discoloring agent, it is clear that there are a small percentage (3 of 34) that contain a different pigment, either by itself or with the hemoglobin. This second pigment has physical characteristics similar to those of material derived from the gut contents of the two aborted fetuses, suggesting fetal "meconium" as the source of the pigment.

On the basis of the analytic studies, discolored amniotic fluid samples can be divided into at least two different groups. Some fluid samples contain blood contamination, whereas in others the discoloration appears to be due to fetal defecation into the amniotic fluid. It does not appear possible to distinguish fluids stained with blood from those stained by intestinal contents either by visual or by spectrophotometric means. It appears that other analytic techniques (SDS electrophoresis, gel chromatography) are necessary to detect the presence of "meconium" in stained amniotic fluid. Although there is little information available about when fetal defecation begins, the work of Abramovich and Gray[13] suggests that the fetus defecates "routinely" until 18 to 20 weeks' gestation, when defecation appears to stop. They suggest that defecation may stop because of innervation of the anal sphincter. If this is the case, one might question the ability of the fetus to defecate in response to stress prior to that time. One could assume that meconium in the amniotic fluid prior to 20 weeks' gestation is just a chance occurrence without prognostic significance. The finding of blood, on the other hand, might portend a poorer prognosis. Further studies are needed to determine the significance of amniotic fluid discolored by meconium in comparison with fluid discolored by blood. In addition, it would be useful to look at the outcome when maternal, in comparison with fetal, blood has stained the fluid. Techniques used in this study did not permit such a distinction to be made.

The mechanism that relates discolored fluid to adverse outcome can only be a subject of hypothesis at this point. If the blood causing discoloration is fetal in origin, subsequent adverse outcomes may be due to the volume of blood lost. If it is maternal, its presence may reflect interference with the fetomaternal circulation by a compromised placenta, which might be due to resolution of bleeding caused by fibrosis or to a partial separation of the placenta after bleeding has occurred into it. It is also possible that such early bleeding might in some way compromise the ability of the fetus to tolerate the stress of labor.

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Discussion

Dr. Richard Allen, Portland, Oregon. Dr. Hanson and colleagues are once again to be congratulated for the excellent research on this interesting and, until recently, seldom-reported phenomenon. Earlier papers on this subject were hampered by small numbers in their series, but with the report in this presentation and that in two other articles in the last year, one by Hankins and colleagues in Dallas and my own article, we now have three large series with fairly comparable results. Most authors found discolored fluid in 1% to 2% of their amniocenteses. Hankins had an incidence of 6.8% with both green and brown fluid. Dr. Hanson's series last year had an incidence of 3.1%, which included transplacental taps 28% of the time—a procedure that one would expect to increase the number of bloody taps. In this article the incidence is 3.3%.

Hankins and colleagues assumed that almost all the discolored fluid samples were discolored by blood, even though most fluid samples were visually green. In conversations that I had with Dr. Hanson last year, we both believed that we could visually distinguish meconium from blood. However, it may well be that we are seeing green fluid in which the discoloration is indeed caused by blood breakdown products. The report by Dr. Hanson and colleagues does have the advantage that "most" of the amniocenteses were done by a single physician (himself).

Dr. Hanson cites a miscarriage rate of 8.2% (9/110), which is slightly higher than the fetal loss rate of 5.06% in my findings. However, the use of 28 weeks as the cutoff definition between miscarriages and premature delivery is somewhat arbitrary. Most tertiary care centers consider delivery at 26 to 28 weeks to result in potentially viable fetuses and not miscarriages. Dr. Hanson's nine cases also include three with abnormal chromosomes; one of these pregnancies was terminated, and one produced an anencephalic twin. I doubt that any of these amniocenteses would have shown discolored fluid for those reasons, per se. Even with the stillborn fetuses, most did *not* have discolored fluid. Therefore it is difficult to conclude that the outcome of these cases had anything to do with the color of the amniotic fluid. In addition, the results of α-fetoprotein testing is not mentioned, but I assume that the level was checked in all cases. Surely the anencephalic twin had an elevated α-fetoprotein level.

In Dr. Hanson's presentation, 62% of those patients with discolored fluid had a history of intrauterine bleeding prior to amniocentesis. I expect that fact to have a significant impact on the findings of discolored fluid. The fact that nine of these patients (13%) had bloody fluid on a repeat tap might also artificially skew the figures, since all of these pregnancies had a normal outcome. I am also puzzled by the fact that in eight of these cases, no fluid at all was obtained on the first tap. I'm sure these taps were all done under the guidance of ultrasonography. Were they done at a very early gestational age?

Since almost all the miscarriages occurred in patients with first-trimester bleeding, Dr. Hanson and colleagues conclude that the patients are at increased risk and need careful monitoring. Although I think we can all agree that any patient with early intrauterine bleeding is at some risk and needs to be monitored more closely, I'm not certain that this bleeding is necessarily related to the color of the fluid. Other factors, such as increased α-fetoprotein levels, may be much more significant. As concluded by myself and others, and as reported by Hankins and colleagues in 1984, "In the absence of an elevated alpha-fetoprotein, the pregnancy outlook is good despite the fact that an intraamniotic hemorrhage has occurred."*

As far as the cause of the discolored fluid is concerned, patients who do have first-trimester bleeding would account for most cases. I would think that this discoloration is almost always maternal, rather than fetal, in origin. In fact, in one of my cases of fetal death the patient later proved to have a large uterine septum on which the placenta was implanted. In addition, some of the cases are iatrogenic (caused by bloody taps and repeat taps), although as Dr. Hanson has previously shown, even transplacental taps should not portend an adverse outcome. In addition, as I pointed out in my article last year and as Dr. Hanson again cites, research by Abramovich and Gray have shown that meconium may be present as a normal physiologic defecation by the fetus in utero.

Last, Dr. Hanson wonders whether early bleeding compromises the ability of the fetus to tolerate the stress of labor, and yet we have no reported losses of term fetuses during labor.

Thus, if there is any relationship between discolored fluid and adverse outcome, in the absence of any other negative factors, it still remains hypothetical at this point in time. Rather, I still believe that we have further evidence, from Dr. Hanson's presentation, that most discolored fluid samples, regardless of whether the discoloration is caused by blood or meconium, do not portend an adverse outcome.

Dr. Robert C. Goodlin, Denver, Colorado. Until a few years ago, I did many amniocenteses, but then I thought that there's more to life [than amniocentesis].

*From Hankins GDV, Rowe J, Quirk JG, Trubey R, Strickland DM. Significance of brown and/or green amniotic fluid at the time of second-trimester genetic amniocentesis. Obstet Gynecol 1984;64:353-8.

However, I never, ever had the low complication rates that Dr. Hanson and colleagues have reported. I'm surprised, however, by their report because I can remember two cases in which the fetus had some abnormality in porphyrin metabolism, and this fluid looked dark and glowed. I can also remember three other cases in which we obtained stained fluid in which level 2 ultrasonography showed large, abdominal fetal wall defects. I believe that sometimes there's much more to stained amniotic fluid than blood and meconium.

Dr. Walter S. Keifer, Seattle, Washington. I'd like to ask Dr. Hanson to define the "Department of Pomology" for the secretary. There may be others here who might be curious also.

Dr. Hanson (Closing). We arbitrarily selected 28 weeks in looking at the miscarriage rate because it's so difficult, as I said last year, to go back into the literature and compare articles on amniocentesis for complications. Everybody has used a different definition, and thus we were being generous. We routinely check α-fetoprotein levels in all our amniotic fluid specimens, and the only patient who had an elevated level was the anencephalic infant, who also had discolored fluid. The one thing I regret is that one of our wells didn't contain α-fetoprotein. We could have then made a statement that there was no fetal blood in any of the discolored fluid.

With regard to not obtaining fluid in some taps, my geographic referral area is very large, extending all the way to the Oregon border and over to Nevada. Many patients at this time of year travel through some hazardous weather conditions and for long distances. I almost always feel obligated to attempt amniocentesis if there is any reasonable chance of obtaining fluid. We're not always successful, and some of these pregnancies are, indeed, at a very early stage. We're performing amniocentesis as early as 12 weeks now; I've even attempted amniocentesis in some 11-week pregnancies. We're getting excellent cell growth and obtaining fluid samples in most of these early amniocenteses. I wish to show that we can approach chorionic villus sampling with regard to the time at which amniocentesis can be performed.

In summary, the one point I definitely want to make here is that you cannot visually distinguish the origin of discoloration of the fluid; when brown is mixed with yellow, green appears in various shades. Spectrophotometric analysis doesn't help distinguish, either. In addition, our studies indicate that it's extremely rare to find meconium in the fluid. Although discolored fluid is a nonspecific finding, I believe it is associated with increased nonspecific morbidity and mortality. I believe that it does behoove the obstetrician to closely follow up on these patients, even though the vast majority will have an excellent pregnancy outcome.

Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist

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A 31-year-old nulligravid patient presented with irregular menses, severe hirsutism, and infertility. Evaluation revealed marked increases of serum androstenedione and testosterone levels and a possible ovarian mass. At operation a cystic teratoma was removed from the left ovary and bilateral wedge resection revealed severe ovarian hyperthecosis. After operation only a transient decrease of androstenedione and testosterone was noted and the patient failed to ovulate or improve clinically. Subsequently a long-acting gonadotropin-releasing hormone agonist was administered daily for 6 months, which reduced circulating Δ^4-steroids and estrogens to levels approximating those of castrated women. Immediately after discontinuation of treatment, ovulation induction was successfully achieved with human menopausal gonadotropin. This report introduces a new therapeutic approach to the problem of severe ovarian hyperthecosis and may provide an opportunity for childbearing in these patients. (Am J Obstet Gynecol 1986;154:1241-8.)

Key words: Hyperthecosis, GnRH agonist, androgen excess, testosterone

The term hyperthecosis was introduced in 1943 to describe isolated nests of luteinized cells distributed throughout the ovarian stroma.[1] In 1949, Culiner and Shippel[2] proposed the syndrome of hyperthecosis, which associated typical pathologic findings with the clinical symptomatology of hyperandrogenism and anovulation. Since recognition of this syndrome, acceptable therapeutic approaches have been difficult to identify and generally have been unsuccessful.

Eradication of androgen excess has been achieved by bilateral oophorectomy but only at the expense of future fertility.[3] In patients desirous of pregnancy, ovulation induction with clomiphene citrate has not been successful and ovarian wedge resection has been attempted. Responses to this procedure appear to reflect the severity of disease. Patients with mild hyperthecosis, characterized by slight elevations of serum testosterone, usually experience transitory reductions in testosterone levels and only temporary resumption of ovulatory function.[4] In more severe cases, manifested by higher elevations of serum testosterone and virilizing signs, ovarian wedge resection also elicits a temporary reduction in testosterone concentrations but fails to induce ovulation.[5] These patients are then faced with progression of masculinization and permanent infertility.

The present report describes a woman with severe androgen excess due to pathologically established ovarian hyperthecosis, in whom surgical management was unsuccessful. Subsequent treatment with a long-acting agonist of gonadotropin-releasing hormone resulted in complete suppression of ovarian androgen production, and immediately after cessation of therapy successful ovulation induction was achieved.

Case report

The patient, a 31-year-old Puerto Rican woman, gravida 0, was referred because of irregular menses and hirsutism. She experienced a normal puberty at age 12 years with regular menses associated with premenstrual symptoms. At that time she had no problem with weight gain or evidence of excessive hair growth. She did not use hormonal medications until age 25. At this point, she married, began to gain weight, and noticed that the menstrual periods were irregular, occurring as frequently as every 2 weeks or as infrequently as every 3 months. Associated with this was the progressive development of hirsutism. She sought medical care, which resulted in the administration of a 3-month

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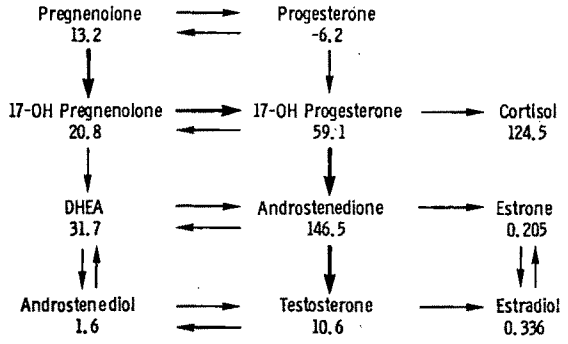


Fig. 1. Schematic pathways of steroid production by hyperthecotic ovaries. The numbers cited represent the mean of ovarian-peripheral gradients in the veins draining each ovary (expressed in nanograms per milliliter).

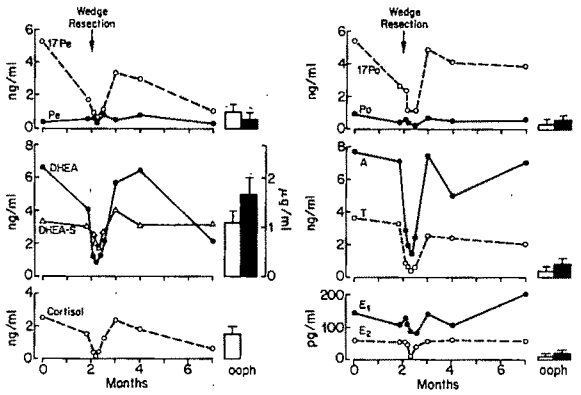


Fig. 2. Serum concentrations of Δ^5- and Δ^4-steroids, cortisol, and estrogens before and after ovarian wedge resection in a patient with severe androgen excess due to hyperthecosis. The mean ± SE values in young castrated women are shown for comparison. Values for dehydroepiandrosterone sulfate are expressed as micrograms per milliliter. *17Pe* = 17-Hydroxypregnenolone; *Pe* = pregnenolone; *DHEA* = dehydroepiandrosterone; *DHEA-S* = dehydroepiandrosterone sulfate; *17Po* = 17-hydroxyprogesterone; *Po* = progesterone; *A* = androstenedione; *T* = testosterone; E_1 = estrone; E_2 = estradiol.

course of oral contraceptives leading to regular withdrawal bleeding. The irregular menstrual intervals returned with discontinuation of therapy.

The physical examination revealed a weight of 168 pounds and a height of 5 feet, 2 inches. Blood pressure was 136/94 mm Hg. The face was rounded and the back had a slight buffalo hump. There was evidence of acanthosis nigricans on the nape of the neck, moderate facial acne, and severe facial hirsutism, including the sides of the face, chin, and upper lip. Severe hair growth was also noted between the breasts. The abdomen was obese with no obvious masses. A male hair pattern with growth up to the umbilicus was present. Pelvic examination revealed a male escutcheon. The base of the clitoris, 8 mm in diameter, was not enlarged. The external genitalia and vagina were normal, and

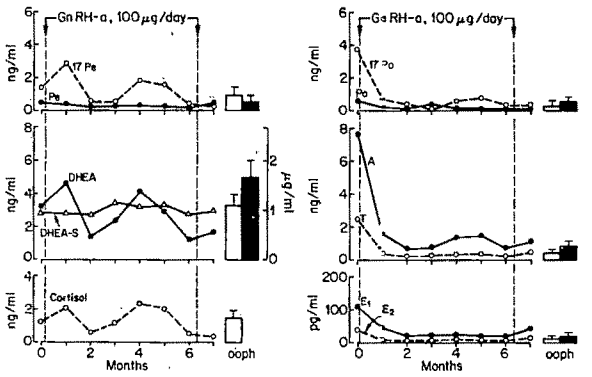


Fig. 3. Serum concentrations of Δ^5- and Δ^4-steroids, cortisol and estrogens before, during, and after gonadotropin-releasing hormone-agonist *(GnRH-a)* administration for 6 months. Values for dehydroepiandrosterone sulfate are expressed as micrograms per milliliter. *17Pe* = 17-Hydroxypregnenolone; *Pe* = pregnenolone; *DHEA* = dehydroepiandrosterone; *DHEA-S* = dehydroepiandrosterone sulfate; *17Po* = 17-hydroxyprogesterone; *Po* = progesterone; *A* = androstenedione; *T* = testosterone; E_1 = estrone; E_2 = estradiol.

the cervix was small. Palpation of the uterus was normal and no adnexal masses were felt.

The screening laboratory evaluation revealed the following: total testosterone, 4.1 ng/ml (range for normal women, 0.1 to 0.55 ng/ml); free testosterone, 110 pg/ml (1 to 6 pg/ml); dehydroepiandrostenedione sulfate, 1.2 μg/ml (0.6 to 2.5 μg/ml); 17-hydroxyprogesterone, 4.6 ng/ml (0.3 to 0.9 ng/ml); cortisol, 237 ng/ml (50 to 250 ng/ml); luteinizing hormone, 16 mIU/ml (5 to 25 mIU/ml); follicle-stimulating hormone, 9 mIU/ml (4 to 20 mIU/ml); and prolactin, 4.0 ng/ml (5 to 20 ng/ml). Administration of dexamethasone, 1 mg, at 11:00 PM resulted in a normal suppression of the fasting morning cortisol level to 10 ng/ml. The corresponding measurement of 17-hydroxyprogesterone was 4.9 ng/ml. In response to subsequent adrenocorticotropic hormone (Cortrosyn, 250 μg intravenously) stimulation, serum 17-hydroxyprogesterone decreased to 3.8 ng/ml.

Ultrasonographic evaluation of the pelvis failed to demonstrate an abnormality. An abdominal computerized tomographic scan revealed bilaterally normal adrenal glands, while the pelvic scan demonstrated a borderline enlarged right ovary and a fat-containing mass in the left adnexa. Retrograde femoral catheterization with selective venous sampling of the left and right adrenal and right ovarian veins was performed and no tissue abnormalities were noted on the fluoroscopic radiograms. Detection of the left ovarian vein was not possible.

On February 6, 1984, the patient underwent exploratory laparotomy with the finding of a benign cystic teratoma (6 by 7 cm) in the left ovary. The remainder of the ovary was elongated, approximately 3 by 5 by 2 cm. The right ovary was cylindrical in shape and measured 3 by 6 by 2 cm. The dermoid cyst was removed

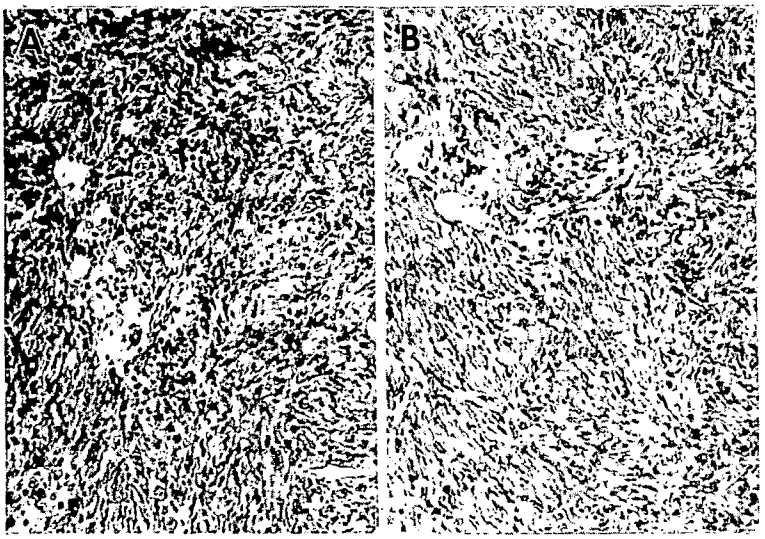


Fig. 4. Routine histologic section of both the right ovary *(a)* and left ovary *(b)* showing abundant and dense stromal cells. Note the focal collections of luteinized cells. (Hematoxylin and eosin. Original magnification ×10.)

from the left ovary, and a bilateral ovarian wedge resection was performed.

Resumption of menses and improvement of hirsutism did not occur during the 5 months after operation. Subsequently, treatment was initiated with a long-acting gonadotropin-releasing hormone agonist, D-His^6(ImBzl),Pro^{9}NEt-gonadotropin-releasing hormone, for 6 months. Two weeks after cessation of therapy, human menopausal gonadotropin was administered for induction of ovulation. A stepwise increasing dose regimen of human menopausal gonadotropin was used until adequate ovarian stimulation was achieved. Administration of three ampules per day for 4 days resulted in a rise of estradiol to 360 pg/ml. This dose was then maintained for another 4 days until serial ultrasound revealed two 14 mm follicles and an estradiol level of 1840 pg/ml. At this time human chorionic gonadotropin, 10,000 IU, was administered intramuscularly. Seven days later the serum progesterone level rose to 30 ng/ml, providing presumptive evidence of ovulation.

Material and methods

During selective venous catheterization, blood samples were obtained from each of the catheterized vessels and a peripheral vein for hormone analysis. Peripheral and left and right ovarian venous blood samples were also collected at the time of operation. Postoperative samples included those obtained consecutively for 4 days and at 1, 2, and 5 months after operation.

Six months after operation, treatment was initiated with the use of the gonadotropin-releasing hormone-agonist 100 μg/day, self-injected subcutaneously for 6 months. The medication was supplied by Ortho Pharmaceutical Corporation, Raritan, New Jersey. Blood samples were obtained before, at monthly intervals during, and at 1 month after cessation of gonadotropin-releasing hormone-agonist therapy. In addition, an endometrial biopsy specimen was obtained before and after treatment.

Serum levels of luteinizing hormone, follicle-stimulating hormone, androstenedione, testosterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, cortisol, estrone, estradiol, progesterone, 17-hydroxyprogesterone, pregnenolone, and 17-hydroxypregnenolone were measured by radioimmunoassay as previously described.[6-10] Reagents for the luteinizing hormone and follicle-stimulating hormone measurements were provided by the National Hormone and Pituitary Program. All luteinizing hormone and follicle-stimulating hormone measurements were converted to milli–international units per milliliter (second international reference preparation). Statistical evaluation was performed by analysis of variance with repeated measures, Student's paired *t* tests, and group *t* tests where appropriate.

Results

Selective venous catheterization. The peripheral, ovarian, and adrenal vein levels of steroids during the selective venous catheterization study were shown in Table I. Peripheral serum concentrations of androstenedione, testosterone, progesterone, and 17-hydroxyprogesterone were elevated above the ranges of values reported for normal women in the early follicular phase of the menstrual cycle. On the basis of ovarian–peripheral venous gradients, the right ovary appeared to actively secrete pregnenolone, 17-hy-

Table I. Steroid hormone concentrations during selective venous catheterization. Peripheral hormone values in normal women are shown for comparison

Vein	*Hormone concentration (ng/ml)*			
	Pregnenolone	*17-Hydroxypregnenolone*	*Progesterone*	*17-Hydroxyprogesterone*
Patients				
Peripheral	1.8	2.4	10.8*	3.5*
Right ovarian	11.8	18.4	8.6	53.8
Left adrenal	18.1	27.9	0.7	71.3
Right adrenal	99.9	384.0	11.7	80.8
Normal women				
Peripheral (mean ± SE)	0.7	1.3	0.4	0.4
	0.1	0.2	0.07	0.04

*$p < 0.05$ in comparison with value in normal women.

droxypregnenolone, 17-hydroxyprogesterone, dehydroepiandrosterone, androstenedione, testosterone, estrone, and estradiol. The largest step-up of androgen in this vein was in androstenedione, which was fifteenfold, compared with that of testosterone and dehydroepiandrosterone, which were increased sevenfold and sixfold, respectively (Fig. 1). In addition, the secretion ratio of androstenedione to testosterone (ovarian–peripheral venous difference of androstenedione divided by that of testosterone) was 14:1. Of the progestins, 17-hydroxyprogesterone displayed the greatest step-up, exceeding that of pregnenolone and 17-hydroxypregnenolone by at least threefold. The ratio of ovarian to peripheral venous concentrations for estrone was 2.5 and for estradiol, 6.4.

A similar pattern of hormone secretion was found in the left adrenal venous sample. There was a minimal gradient of cortisol. The right adrenal vein exhibited marked adrenal–peripheral venous gradients for all steroids except estrone and estradiol. In particular large gradients of pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, and cortisol were noted. Based on these findings, the pattern of hormone secretion by the left adrenal gland was very different from that of the right adrenal and was similar to that of the right ovary.

Intraoperative studies. Table II shows the levels of androgens found in the peripheral and ovarian venous samples obtained at operation. Based on the peripheral–ovarian venous gradients, the patterns of androgen secretion were similar for the two ovaries.

Response to operation. Circulating steroid hormone concentrations before, during, and after ovarian wedge resection are shown in Fig. 2. Mean ± SE values found in 10 women between 20 and 30 years of age who had undergone bilateral oophorectomy for gynecologic disease are shown for reference. In response to operation, there were immediate decreases of most Δ^5,- and Δ^4-steroids, cortisol, estrone, and estradiol, with a nadir reached on postoperative day 3. These included decreases of elevated androstenedione, testosterone, and 17-hydroxyprogesterone levels into the range of values for normal women. Serum levels of pregnenolone and progesterone were unaltered. Shortly after these declines, progressive rises were observed for all suppressed steroids, which resulted in the return of concentrations to preoperative values within 4 weeks after operation. During the next 4 months, circulating steroid levels remained unchanged.

Response to gonadotropin-releasing hormone-agonist. As shown in Fig. 3, daily administration of a gonadotropin-releasing hormone-agonist had a significant suppressive effect on all Δ^4-steroids, estrone and estradiol. By 2 months of treatment, the circulating levels of these were similar to those of surgically castrated women. In contrast, the Δ^5-steroids and cortisol were not affected by the gonadotropin-releasing hormone-agonist. The decremental change of Δ^4-steroids and estrogens in response to the gonadotropin-releasing hormone-agonist was more pronounced than that observed after ovarian wedge resection. Inhibition of ovarian steroidogenesis persisted for the 6 months of gonadotropin-releasing hormone-agonist administration and for 1 month thereafter.

Basal serum luteinizing hormone and follicle-stimulating hormone levels were 13 and 9 mIU/ml, respectively. These concentrations remained constant throughout the course of gonadotropin-releasing hormone-agonist treatment as the mean (±SE) value for luteinizing hormone was 14.7 ± 0.7 mIU/ml and for follicle-stimulating hormone, 8.7 ± 0.4 mIU/ml.

Pathologic findings. Ovarian tissue from wedge resection was firm and yellow-tan in appearance. Histologically, the tunica albuginea was thickened and follicles were seen in various stages of development. In both ovaries stromal cells were abundant, dense, and spindle-shaped without nuclear abnormalities (Fig. 4). Focal collections of luteinized cells were also noted. Reactive luteinization of tissue adjacent to the teratoma was not found. In the right ovary there was an involuting corpus luteum.

Hormone concentrations (ng/ml)						
Dehydroepiandrosterone	*Androstenediol*	*Cortisol*	*Androstenedione*	*Testosterone*	*Estrone*	*Estradiol*
5.8	1.4	179	8.4*	1.7*	0.13	0.06
20.3	2.5	263	126 8	10.8	0.34	0.39
54.7	3.5	344	182.9	13.7	0.33	0.41
419.3	15.3	2530	143.0	4.3	0.22	0.06
5.1	0.9	195	0.9	0.3	0.05	0.06
0.6	0.1	19	0.06	0.02	0.01	0.01

Table II. Intraoperative peripheral and ovarian venous androgen concentrations

	Androgen concentration (ng/ml)			
Vein	*Androstenedione*	*Testosterone*	*Dehydroepiandrosterone*	*Dehydroepiandrosterone sulfate*
Peripheral	7.1	3.3	4.1	1020
Left ovarian	301.9	56.0	74.2	1430
Right ovarian	190.2	35.3	53.4	1480

Tissue specimens obtained at endometrial biopsy revealed hyperplastic endometrium before gonadotropin-releasing hormone-agonist administration. After 6 months of treatment, repeat biopsy demonstrated atrophic endometrial glands and stroma.

Comment

On the basis of the marked elevation of the testosterone level (4.1 ng/ml) in the screening blood sample, the normal suppression of serum cortisol with dexamethasone administration, and the limited response of 17-hydroxyprogesterone to adrenocorticotropic hormone administration, it was concluded during the patient's initial evaluation that she had either an androgen-secreting tumor of the ovary or of the adrenal gland or ovarian hyperthecosis. These conditions are generally associated with testosterone elevations of this magnitude, particularly in the absence of hypercortisolism or a 21-hydroxylase defect of the adrenal glands.

Ultrasonic evaluation of the pelvis was not helpful in localizing the site of the abnormal hormone secretion. Computerized tomographic scan of the abdomen and pelvis suggested the source may have been the left ovary. Retrograde venous sampling and measurement of hormonal gradients between the peripheral and adrenal and ovarian veins were not helpful because of the inability to catheterize the left ovarian vein. This difficulty was surprising since entry of this vessel into the left renal vein is usually easy to identify.[11] The fact that the profile of hormones found in the left adrenal vein was similar to the pattern observed in the right ovarian but not the right adrenal vein suggests that the left ovarian vein likely had an aberrant connection with the left adrenal vein. This conclusion is supported by the absence of a step-up of cortisol in the left adrenal sample and the finding of similar androgen levels in the left and right ovarian venous samples obtained at operation.

We have reported the use of retrograde catheterization to localize androgen-secreting tumors on several occasions and have had excellent success with this method.[11] The present results indicate that this technique has potential limitations because of the variable anatomy of the venous systems draining these glands.

At the time of operation two ovarian lesions were observed, a benign cystic teratoma and ovarian hyperthecosis. Although both lesions have been associated with marked androgen excess, it is likely the hyperthecosis was responsible in this patient since removal of the ovarian tumor was associated with only a transitory reduction of androgens.

The implied functional nature of this disorder suggests that successful therapy might be achieved by suppression of androgen production instead of extirpation of the ovary. However, previous attempts at medical management have been disappointing and the current recommended treatment remains either ovarian wedge resection or bilateral oophorectomy. Wedge resection has been used with satisfactory results in patients with the mild form of hyperthecosis as characterized by normal or slightly elevated testosterone levels. In five patients with mild disease, ovarian wedge resection resulted in a return of cyclic menses in all and pregnancy in one.[12] The highest circulating concentration of testosterone in these patients was 0.72 ng/ml. Unfortunately most studies have not included long-

term follow-up and therefore ultimate outcome has been undeterminable. Women with higher serum testosterone levels are more refractory to this type of treatment. Some reports have documented initial ovulatory responses after operation, which have not persisted over time.[5] In most instances, the serum testosterone level ranged between 1 and 3 ng/ml.

Our patient exhibited severe androgen excess and, similar to the experience of others, reverted to her preoperative condition soon after wedge resection. From these data it would appear that the level of testosterone correlates not only with the degree of clinical symptoms but also with the response to surgical therapy. The transitory fall of both Δ^5- and Δ^4-steroids at the time of operation presumably reflected the impact of anesthesia and operation on adrenal steroid secretion coupled with an effect of wedge resection on ovarian steroidogenesis.

Previously we have shown that the administration of a gonadotropin-releasing hormone-agonist to women with polycystic ovary syndrome and ovulatory women with endometriosis resulted in complete suppression of ovarian steroid secretion.[13] The unique ability of this drug to cause a medical oophorectomy compelled us to implement its use in our patient. Consistent with our earlier experience, profund reductions of ovarian androgen, estrogen, and progestin secretion were observed. After 1 month of treatment, serum androstenedione and testosterone levels approximated concentrations seen in normal premenopausal women and by 2 months both androgens were in the range seen in castrated women. That this response was somewhat delayed compared with that seen in women with polycystic ovary syndrome may reflect the severity of disease as the pretreatment circulating levels of androstenedione and testosterone far exceeded those seen in patients with polycystic ovary syndrome. On the other hand, measurements of these hormones at 1 month as well as at 4 and 5 months of treatment may have reflected spontaneous adrenal activity that contributed to increased serum concentrations as simultaneous increases were noted for cortisol and the Δ^5-steroids dehydroepiandrosterone and 17-hydroxypregnenolone. In general, adrenal androgens were not significantly altered by gonadotropin-releasing hormone-agonist therapy.

Clinically, the patient did not perceive any change of hair growth pattern during gonadotropin-releasing hormone-agonist treatment. This is not surprising since severe hirsutism has typically been slow to respond to a variety of therapeutic measures including administration of antiandrogens such as cyproterone acetate. The duration of ovarian androgen suppression was also only 4 months and the facial hair growth cycle exceeds at least 6 months. In comparison, the reduction of estrogens was probably responsible for conversion of a hyperplastic endometrium to an atrophic one.

One of the major problems confronting our patient was the inability to conceive. The successful ovulation induction was achieved in our patient by human menopausal gonadotropin administration in the immediate posttreatment period suggests that elimination of the enormous amounts of intraovarian androgen by the gonadotropin-releasing hormone-agonist may have been instrumental. Previous studies have shown that local androgen production interferes with follicular growth and development. In hypophysectomized immature female rats the ovarian weight response to diethylstilbestrol is inhibited by human chorionic gonadotropin. This effect is reversed by administration of an antiandrogen. In the same rat model, granulosa cell death and follicular attrition of medium-sized follicles may be accelerated by the addition of dihydrotestosterone or human chorionic gonadotropin treatment. These findings support the observation in humans that hyperandrogenic ovaries are often associated with multiple follicular cysts, which are degenerative and lack healthy-appearing oocytes.[14]

Similar to the reports of several prior studies,[4, 5] circulating luteinizing hormone levels were normal in our patient, suggesting greater responsiveness of ovarian stromal cells to luteinizing hormone relative to an absolute increase in number and/or enhanced sensitivity of individual cells. Another mechanism could be inferred from the ovarian response to the gonadotropin-releasing hormone-agonist. Since we have demonstrated previously a marked suppression of bioactive luteinizing hormone concentrations in women with both endometriosis and polycystic ovary syndrome treated with a gonadotropin-releasing hormone-agonist,[15] it was likely that bioactive luteinizing hormone was suppressed in our patient during therapy, thereby accounting for the reduction of ovarian androgen. These data also support a primary role for luteinizing hormone in the generation of excess ovarian androgen in this syndrome.

With the dominant ratio method, the preferred steroidogenic pathway of these hyperthecotic ovaries was pregnenolone → 17-hydroxypregnenolone → 17-hydroxyprogesterone → androstenedione → testosterone (Fig. 1). These results differ from those of Abraham and Buster,[16] who reported two cases of hyperthecosis and normal circulating androstenedione levels. They proposed a selective blockade of $\Delta^4 C_{17\text{-}20}$ desmolase to account for the normal serum androstenedione values and a preferential pregnenolone → 17-hydroxypregnenolone → dehydroepiandrosterone → androstenediol → testosterone pathway to explain the elevated serum testosterone concentrations. In our patient there were step-ups of 17-hydroxypregnenolone → dehydroepiandrosterone and 17-hydroxyprogesterone → androstenedione in the ovarian veins, indicating intact $C_{17\text{-}20}$ desmolase activity. In addition, we

were unable to establish significant ovarian cortisol secretion, as noted by Abraham and Buster,[16] compared with the large right adrenal–peripheral venous gradient for this hormone.

In summary, this case report has demonstrated the dramatic suppressive effect of a gonadotropin-releasing hormone-agonist on ovarian steroidogenesis and androgen production in severe hyperthecosis. The lack of symptomatic improvement of hirsutism was probably related to the short interval of treatment and suggests the need for more prolonged therapy in these patients. These data indicate that for the first time a method is available that can eliminate the massive androgen secretion seen in patients with this disease without a need for bilateral oophorectomy. It is likely that the androgen excess in these patients could be controlled by this method until childbearing is completed before the performance of ovarian extirpation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Charles D. Kimball, Seattle, Washington. I am very pleased to have the opportunity to comment on Dr. Judd's article. I hope to raise a few logical questions and suggest some possible answers.

This report is an excellent example of a case study that integrates basic science research with clinical practice of medicine. For more than a decade Dr. Judd and his colleagues have made impressive and significant contributions to our understanding of androgenizing endocrine disorders of women. This report clearly demonstrates the fact that complete but reversible castration can be accomplished by physiologic rather than surgical or pharmacologic means, which in my view is a new peak in therapeutic achievement. It also shows that exogenous gonadotropes can rehabilitate ovaries that are advanced in hyperthecosis.

The elevated androgenic production by the theca cells of this patient beginning 12 years after menarche and shortly after marriage raises interesting and exciting questions for future research regarding the molecular mechanisms that triggered the prolonged and persistent luteinizing hormone stimulation of the androgenic hormones. It also opens the way for consideration of the role of opioid and catecholamine hormones in modulating the periodic oscillatory frequency of gonadotropin-releasing hormone output by hypothalamic cells and the adjacent neurosecretory cells that produce β-endorphin and dopamine. It appears that the ovarian cycling that was normal in this patient before the onset of virilization got "stuck" in the "groove" and repeated the endorphin-controlled gonadotropin-releasing hormone pulse frequency that evoked excessive luteinizing hormone production.

There are also questions about the enzyme mechanisms that demethylate and aromatize androgens to estrogens. Failure of this enzyme mechanism would result in a vicious feedback cycle whereby high steroid-dependent opioid hormone levels sustain the prolonged and excessive gonadotropin-releasing hormone–luteinizing hormone–C^{19}-steroid output and thus prevent normal maturation and lysis of the corpus luteum with collapse of the mutually dependent opioid-steroid structure.

There are interesting questions regarding the function of catecholamines, especially dopamine, in the ovulation cycle of this patient. Numerous reports have indicated that β-endorphin and dopamine are secreted in counterbalance with each other in the hypothalamic-hypophyseal portal system that regulates prolactin, follicle-stimulating hormone, and luteinizing hormone production.

Elevated β-endorphin serum levels have recently been reported in amenorrheic, hirsute, hyperandro-

genic, obese women.[1,2] Serum β-endorphin and its precursor polypeptide β-lipotropin are split by enzymatic cleavage from the precursor protein proopiomelanocortin in response to physiologic stress. One wonders if a stressful marriage might have produced chronic elevation of β-lipotropin and anomalous cleavage by enzymes that liberate melanocyte-stimulating hormone as well as excess β-endorphin. We have two ready clues to this possible mechanism. The earlier article by Judd et al. on familial hyperthecosis indicates a genetic tendency (see reference 5 of article). The other is the presence of acanthosis nigricans, indicating a melanocyte-stimulating hormone effect on pineal hormones that also regulate estrus and the mating season. In other words, this patient may not have been sexually ready, which many women equate with stress.

Many intriguing lines of basic science investigation are opened by case studies such as this, which demonstrate important benefits from integrating clinical research and clinical practice.

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Dr. Frederick Hanson, Sacramento, California. With the use of feralin acetate for endometriosis, we are concerned with osteoporosis, and some of the bone densitometric studies in that part of our protocol seem to indicate that this is a potential problem. Did you look at this in this woman, as it will be a problem with use of these drugs? If a patient does not desire pregnancy, what do you see as long-term therapy? I assume rebound will occur and she will be right back where she was as soon as the agonist is stopped. Since we cannot use these drugs on a long-term basis what are her other options? She cannot be pregnant all the time either.

Dr. Simon Henderson, San Francisco, California. I would like to ask just one or two questions and first of all just start with my assumption that this was essentially a patient who was trying to become pregnant but was also a research patient and that is why the analogue was given. I understand also that you suspected an ovarian mass, and maybe that was why you performed the wedge resection. I was not totally clear from all of this what your plan of practical management would be for these patients in the future. We do have other modalities such as using menotropins (gonadotropin), without many of the procedures performed in this patient. Also clomiphene sometimes works in these patients. I would like your comments as to the basic core management of these patients in the future.

Dr. Jack Hallatt, Los Angeles, California. We have made a striking observation that there is a correlation between the incidence of dermoid cysts and polycystic ovary syndrome. Now, is this a true relationship or is it just anecdotal and coincidental?

Dr. Judd (Closing). Dr. Kimball addressed the issue of the mechanism by which this patient had elevated androgen levels. It is very clear that the elevation was produced by the gonadotropins. This is based on the observation that when we suppressed the gonadotropins the androgen level decreased. However, the gonadotropin levels were repetitively measured in the normal range. So either she has a group of cells that are hypersensitive to gonadotropins or she has a group of cells that have developed on their own and will now be sustained by normal gonadotropin levels.

Dr. Hanson raised a question in regard to long-term use of the gonadotropin-releasing hormone analogue. In a patient with severe hyperthecosis who is trying to become pregnant, it is very likely that the only way this can be accomplished is by suppression of ovarian function. There is no reason in a case such as this that long-term suppression should not be used. Also there is absolutely no reason not to administer hormone replacement until the patients are ready to have children. Only after the family is completed would ovarian removal need to be considered. Until now this has not been possible. Use of the analogue provides us with a way of temporizing. As to the issue of long-term analogue use in osteoporosis, I do not think this is a problem with severe hyperthecosis. In this case there is absolutely no reason not to give estrogens and progesterone during analogue administration.

Dr. Henderson brought up the concept that there are other ways of treating this disorder. I would disagree with you, Dr. Henderson. The track record in the literature is that wedge resection, clomiphene, Pergonal, and any form of therapy other than bilateral oophorectomy have not been adequate to treat this hyperandrogenism. Consequently, in treating women who have androgen levels in the male range, other forms of therapy will have to be realized. This is the first time there is an alternative other than surgical oophorectomy.

Dr. Hallatt brought up the very interesting question of the possible relationship between dermoid cysts and polycystic ovary syndrome. The fact is that there is a potential relationship. Dermoids and a variety of other tumors of the ovary have been associated with increased androgen production. The tumors themselves do not seem to produce the androgens. What happens is that the tumors are probably producing a human chorionic gonadotropin–like substance. At the local level they stimulate stromal hypertrophy and luteinization of the stroma immediately adjacent to the tumor. This stroma produces the increased androgen levels, as has been shown in patients with dermoid cysts. As ovarian androgen production is increased, it is possible that polycystic ovarian disease develops We do not think this happened in our patient for two reasons: First, the hyperthecosis was bilateral, and this has never been described with a tumor. The stromal hyperplasia has been described only in the immediate vicinity of the tumor. Second, we removed the tumor and the condition persisted.

Chorionic villus sampling: Experience of the first 1000 cases

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Chorionic villus sampling has become widely accepted as a first-trimester alternative to amniocentesis for the prenatal detection of genetic disorders. Our experience with 1000 consecutive cases suggests that spontaneous abortion following the procedure is related to the gestational age at which it is performed, and the rate is acceptably low (3.8%) when the procedure is performed from the ninth through the eleventh menstrual week. Later complications include chorioamnionitis (0.6%) and delayed rupture of membranes and/or oligohydramnios (0.8%). A discrepancy between the villus karyotype and that of the fetus was found in 1.7% of cases and most commonly consisted of mosaicism in the villus sample for a chromosomal abnormality that was not found in fetal samples. We conclude that although chorionic villus sampling appears to be an acceptably safe and reliable procedure, further investigation is needed before this first-trimester technique is widely utilized as an alternative to amniocentesis. (AM J OBSTET GYNECOL 1986;154:1249-52.)

Key words: Chorionic villus sampling, complications, karyotype, amniocentesis

In the less than 5 years since the widespread introduction of chorionic villus sampling as a clinical diagnostic tool, the procedure has emerged as a first-trimester alternative to amniocentesis. A number of previous reports based on small numbers of patients with limited follow-up have indicated the feasibility of chorionic villus sampling and have provided some preliminary information on the safety, efficacy, and reliability of sampling in the first trimester.[1-3] Our experience with the first 1000 consecutive chorionic villus samplings performed at the University of California, San Francisco, is presented to more specifically identify those factors that influence the safety and reliability of chorionic villus sampling.

Methods

The chorionic villus sampling program at the University of California, San Francisco, has been previously described.[3] Briefly, sampling was performed between 8 and 12 weeks from the last menstrual period with the use of a 16-gauge Intracath (Deseret Co., Sandy, Utah). The catheter was directed transcervically under sonographic guidance to a location within the villus of the chorion frondosum. Villus samples of 5 to 50 mg (wet weight) were obtained by suction aspiration. For cytogenetic analysis, long-term cultures were established in duplicate in Chang's "closed" medium (Hana Biologics, Berkeley, California). All procedures were performed under a protocol approved by the University of California, San Francisco, Committee on Human Research.

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Results

Table I outlines the indications for chorionic villus sampling and the number of abnormalities found. As is the case for amniocentesis, over 90% of the procedures were performed for advanced maternal age. The incidence of chromosomal abnormality (4.2%) in this group is appreciably higher than that found in the second trimester (2.5%).[4] This difference indicates that a significant proportion of chromosomally abnormal fetuses are lost before the sixteenth week of pregnancy.

The categories of chromosomal abnormalities are given in Table II. As expected, the most common finding is autosomal trisomy, accounting for almost 50% of the abnormalities detected. A disturbing finding is the 1.2% incidence of mosaicism, which represents almost one fourth of the abnormalities seen. A detailed analysis of these cases is shown in Tables III and IV. Most of these karyotypes would be of clinical significance if detected at amniocentesis. With the exception of the one case of mosaicism for trisomy 21, which almost certainly represented a low level of maternal cell contamination, none of the villus karyotypic abnormalities were confirmed by amniocentesis or fetal tissue studies. Despite the small number of cases, it is notable that 33% of the patients with villus chromosomal mosaicism who intended to continue their pregnancies spontaneously aborted. Only one of these patients has completed her pregnancy at the time of this writing, and she was delivered of a 3260 gm healthy male infant.

There were two additional cases in which a discrepancy was noted between the villus karyotype and that of the fetus. These two instances of chromosomal aneu-

Table I. Indications for chorionic villus sampling

Indications	*No. sampled*	*Abnormal samples*	
		No.	*%*
Maternal age	899	38	4.2
Other chromosomal	45	6	13.3
X-linked disorders	24	8	33.3
Biochemical	15	3	20.0
Total	983*	55	5.6

*Seventeen patients with unsuccessful sampling or culture.

ploidy (45,X in one case; 47,XX, + 16 in the other) were not confirmed on fetal skin samples (46,XY and 46,XX, respectively) obtained following pregnancy termination. Unfortunately, no placental tissue was submitted for analysis.

Complications. Immediate complications of chorionic villus sampling are uncommon. An adequate sample was obtained on the first visit in 97.4% of the cases. There were only two cases of rupture of the amnion at the time of the procedure. We consider culture failure as an immediate complication, and it is likewise uncommon, occurring in less than 1% of the cases.

The major short-term complications of chorionic villus sampling are pregnancy loss and diagnostic error. In addition to those cases noted above, there were three cases of diagnostic error caused by maternal cell contamination. All three errors were detected and corrected prior to 20 menstrual weeks. The overall error rate (both false positive and false negative results) was 1.7%.

Details of the pregnancy losses are given in Table V. Spontaneous abortion followed the procedure in 51 cases (5.4%), three of which were attributed to chorioamnionitis. Another three cases of acute chorioamnionitis were diagnosed after 20 menstrual weeks.

There were three instances of delayed rupture of the fetal membranes, each occurring approximately 8 weeks after the procedure. In another five cases, severe oligohydramnios was noted on sonograms obtained between 16 and 20 weeks' gestation. Despite the finding of almost a complete lack of amniotic fluid, there was no clinical history to suggest overt rupture of the membranes or leakage of amniotic fluid. No gross fetal anomalies were noted in these pregnancies, which either spontaneously aborted (four cases) or were electively terminated (two cases).

In Table VI the 950 pregnancies meant to continue are divided on the basis of gestational age at the time of chorionic villus sampling. The incidence of spontaneous abortion in those patients sampled from 9 menstrual weeks to 11 weeks 6 days was only 3.8%. On the basis of relatively small numbers, patients having chorionic villus sampling prior to and after this period had an appreciably higher loss rate (12.9% and 17.1%, respectively).

Table II. Chromosomal abnormalities

Trisomies	23
Mosaicism	12
45,X	5
Translocation—unbalanced	6
Translocation—unbalanced (de novo)	3
Chromosomal fragment	1
Total	50

Long-term complications of chorionic villus sampling theoretically could include fetal anomalies, placental accidents or dysfunction, and intrauterine growth retardation. Approximately 50% of those pregnancies that were intended to be continued have now resulted in delivery. Four infants have been born with congenital anomalies: one with Goldenhar syndrome (hemifacial microsomia), one with severe craniosynostosis resulting in a cloverleaf skull deformity, one with achondroplasia (both parents have normal stature), and one with bilateral polydactyly. None of these anomalies appears to be related to the prenatal diagnostic procedure, and this incidence is no different from what would be expected in the general population. There have been two cases of placenta previa and no instances of intrauterine growth retardation, although ascertainment is not yet complete.

Comment

Chorionic villus sampling has rapidly gained wide acceptance as an alternative to amniocentesis for the prenatal diagnosis of genetic disorders. Although it is being performed in many centers throughout the world, only preliminary evidence is available regarding the safety, efficacy, and reliability of this new procedure. Based on our experience with 1000 samplings, it appears that chorionic villus sampling is acceptably safe and reliable. However, several issues are raised that must be further evaluated before chorionic villus sampling can be widely applied for prenatal diagnosis.

There is a significant incidence (1.7%) of discrepancy between the apparent karyotype of the villi and that of the fetus. A small portion of this error rate is attributable to maternal cell contamination, which appears to be related to experience. With the use of meticulous techniques for removing maternal cells from the villus samples, this source of error should be small. The remainder of the cases appear to represent true mosaicism within the placenta. That this finding is biologic, and not simply an artifact of long-term culture, is supported by studies of spontaneous abortion material in which mosaic trisomies occur in a significant proportion of cases.[5] These abortion analyses are commonly done on extraembryonic tissue, not on fetal material, and

Table III. Mosaicism in chorionic villus sampling cultures—autosomal

Villus karyotype	*Amniocentesis*	*Pregnancy outcome*	*Confirmation*
46,XX/46,XX,t(8;13)	Not done	Unadvised TAB	Not obtained
46,XX/47,XX,+18	Not done	Unadvised TAB	46,XX
46,XX/47,XX,+21	Not done	TAB	47,XX,+21
46,XY/47,XY,+18	46,XY	SAB after amniocentesis	Not obtained
46,XY/46,XY,8q+	Not done	SAB after CVS	Not obtained
46,XX/47,XX,+15	46,XX	Delivered (4400 gm)	Pending
46,XY/47,XY,+8	46,XY	Ongoing	—

TAB = Therapeutic abortion; SAB = spontaneous abortion; CVS = chorionic villus sampling.

Table IV. Mosaicism in chorionic villus sampling cultures—sex chromosomes

Villus karyotype	*Amniocentesis*	*Pregnancy outcome*	*Confirmation*
46,XX/45,X	46,XX	Missed abortion noted at time of amniocentesis	No growth
46,XY/45,X	46,XY	Delivered (3270 gm)	46,XY
46,XY/45,X	46,XY	Delivered (3610 gm)	Pending
46,XX/45,X/45,X,10q+/45,Xdel(10q)	46,XX	Delivered (3290 gm)	Pending
46,XY/45,X	46,XY	SAB after amniocentesis	Pending

Table V. Pregnancy loss summary

Type	*No.*	*%*
Spontaneous abortion (<20 wk)	48	5.1
Premature labor	4	0.4
Chorioamnionitis (<20 wk)	3	0.3
Chorioamnionitis (>20 wk)	3	0.3
Delayed rupture of membranes	3	0.3
Severe oligohydramnios	5	0.5
Total	66	7.0

Table VI. Menstrual age of patients at total chorionic villus sampling and chromosomally normal spontaneous abortion

	Overall		
		SAB	
Gestational age	*Total**	*No.*	*%*
$<9^0$	116	15	12.9
9^0 to 11^6	799	30	3.8
$\geq 12^0$	35	6	17.1
Total	950	51	5.4

SAB = Spontaneous abortion.

*Patients having therapeutic abortions have been removed from the calculations.

they suggest that the rates for aneuploidy in spontaneous abortion would be lower if fetal tissue only were evaluated. The significance of chromosomal abnormality limited to extraembryonic tissue is not yet clear. A report by Kalousek and Dill[6] suggested there may be an association between chromosomal mosaicism in the placenta and intrauterine growth retardation. Because of the uncertain significance of mosaicism in the villus sample, we offer these patients a follow-up amniocentesis. The results of the amniotic fluid cell analysis have been normal in all cases to date, and there are a number of ongoing pregnancies that should provide some preliminary information to help answer these questions.

Previous reports from this institution have suggested that infection may be a small but important aspect of the complications of chorionic villus sampling.[7,8] There remains a significant rate of chorioamnionitis (0.6%), and, as shown previously, there is a clinical history to suggest infection in a large portion of patients having spontaneous abortion after chorionic villus sampling.[7]

A striking finding in this study is severe oligohydramnios in six patients, none of whom had a history of fluid leakage. This suggests that chorionic villus sampling may in some way damage the chorion and/or amnion and may alter the normal fetomaternal fluid dynamics. If to these six cases are added those with both immediate and delayed rupture of the amnion, there is a relatively high incidence (1%) of complications related to membrane damage. However, these complications can occur in pregnancies that have not had prenatal testing, and only with a controlled study can the true incidence related to chorionic villus sampling be determined.

Although there does appear to be a small risk of later complications related to chorionic villus sampling, our spontaneous abortion rate of 3.8% is not different from those rates reported in sonographically normal pregnancies of comparable gestation and maternal age.[9,10] We must emphasize that this low rate occurs in those cases in which chorionic villus sampling was done after 8 menstrual weeks and prior to 12 weeks.

In summary, our experience suggests that chorionic villus sampling is an acceptably safe and reliable method of prenatal diagnosis. However, the questions raised regarding infection, membrane damage, and placental mosaicism must be answered. The ongoing National Institutes of Health collaborative study of both chorionic villus sampling patients and control patients undergoing amniocentesis should provide these answers.

The technical assistance of Alison Porter-Stein and Timothy Tomashek, the genetic counseling assistance of Joan Hogge and Roberta Tung, and the sonographic assistance of Antoinette Golbus and Helen Gammon are appreciated. Without them, this program would not be possible. Dr. Gary Simpson kindly provided ongoing discussion, suggestions, and technical assistance in the development of the program.

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Detection of preterm labor by ambulatory monitoring of uterine activity for the management of oral tocolysis

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A study to evaluate whether ambulatory tocodynamometry at home could enhance the management of oral tocolysis was performed. On discharge from the hospital, after completing parenteral tocolysis, 60 patients received a lightweight tocodynamometer, designed for storage and transmission of uterine activity data. Sixty additional contemporary patients, who were matched for maternal age, parity, and risk factors, served as a control group. In addition to instructions regarding self-detection of recurrent preterm labor, monitored patients recorded uterine activity for ≥200 min/day and then transmitted the data to the study center. Tocolytic dosage was adjusted to maintain mean uterine contraction frequency of <4/hr. Persistent uterine contractions of ≥4/hr lead to in-hospital evaluation for recurrent preterm labor. The results indicate that the monitored group had a lower incidence of preterm births mostly because of a decrease in the proportion of patients with failed tocolysis. It is suggested that surveillance of uterine activity at home during oral treatment for preterm labor may be instrumental in improving perinatal outcome in high-risk patients. (Am J Obstet Gynecol 1986;154:1253-6.)

Key words: Labor, preterm, oral tocolysis, tocodynamometry, perinatal outcome, management, home

Currently, the management of parenteral tocolytic therapy relies mostly on uterine activity response to treatment, a response that is best evaluated by external tocodynamometry. Once successful in achieving adequate control of contractions, parenteral tocolysis is replaced by an oral maintenance dose.[1-3] However, the effect of this tocolytic regimen on uterine activity at home can no longer be objectively evaluated even though adjustments in treatment may be required. It is thus conceivable that if means to follow uterine activity response to oral tocolysis at home were available, proper adjustments in treatment could be made and thereby enhance the effectiveness of tocolysis. If in spite of these adjustments oral tocolysis can no longer control uterine activity, home monitoring could help make the early diagnosis of recurrent preterm labor. The following study evaluated whether ambulatory tocodynamometry at home could contribute to the management of patients who were receiving oral tocolysis after their initial preterm labor episode had been controlled by parenteral in-hospital therapy.

Material and methods

One hundred twenty patients who completed their initial in-hospital course of parenteral tocolysis and were about to be discharged home with oral medication were recruited into the study after signing the appropriate consent forms. The initial diagnosis of preterm labor was made whenever persistent and regular uterine contractions were associated with progressive cervical dilation and effacement before 36 completed gestational weeks. The recurrence of such episodes in these patients was termed recurrent preterm labor. Gestational age was determined by considering the last menstrual period, uterine size, sonography, and neonatal evaluation. A delivery before 36 completed weeks' gestation is reported here as preterm. A delivery before 36 weeks' gestation that was the result of a condition such as severe preeclampsia or fetal death is reported here as an indicated preterm birth.

Sixty of the patients (group 1) received a lightweight uterine activity monitoring device designed for ambulatory recording, storage, and transmission of uterine activity data.[4] Because of the limited number of monitoring devices, assignment to the monitored group was not at random but rather on an availability basis. All contemporary patients who were eligible for home monitoring, but for whom no monitor was available, formed a pool from which 60 control subjects (group 2) were selected to match the following variables in group 1: (1) maternal age (±5 years), (2) parity, and (3) risk factors (for instance, multifetal gestations, previous preterm births). Only patients who started prenatal care at or before 12 weeks' gestation and who were cared for by their private obstetricians throughout pregnancy were included. Patients who were transferred into this institution with recurrent preterm labor

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Table I. Maternal risk factors for preterm birth

Risk factor	Monitored		Matched	
	n	%	*n*	%
Multifetal gestation	20	33	9	15
Previous preterm birth				
1	18	30	26	43
>1	8	13	5	8
Diethylstilbestrol, cerclage, uterine anomaly	6	10	4	7
No apparent risk factor	8	13	16	27

and then delivered before term and those who received tocolytics not in compliance with the earlier described definition of preterm labor were not included in the study. In spite of attempts at exact matching, there were not enough multifetal gestations available for the control group, and these were therefore substituted by patients with other risk factors for preterm labor and delivery. As shown in Table I, the monitored group included a somewhat larger proportion of patients at high risk of preterm birth. On discharge from the hospital each study patient in both groups received instructions about the following: signs and symptoms of recurrent preterm labor,[5, 6] techniques of self-palpation for excessive contractions,[5, 6] and techniques of determining pulse rate. Patients were also instructed to remain at bed rest except for limited ambulation (meals and the bathroom).

The 60 monitored patients in group 1 recorded uterine activity three or four times daily for a cumulative total of 200 min/24 hr. The stored data were then transmitted daily, via the telephone, to receiving tocographs, which were located in the study center. Each transmission lasted 6 to 8 minutes and verbal feedback about the findings was given to the subjects. Additional transmissions were done if so desired by the investigators and an open emergency line was available 24 hours a day for monitored patients who wished to transmit additional data. Only contractions lasting more than 35 seconds were included in the analysis.[7] Shorter-duration contractions, most of which were of the low-amplitude, high-frequency type ("Alvarez waves"), were ignored. If four or more contractions per hour were seen, oral tocolysis was increased (dosage and/or frequency) to maintain uterine activity at a frequency of ≤3/hr while at bed rest. If the patients' pulse rates, just before taking the next dosage, exceeded 110 bpm and uterine activity persisted at four or more contractions per hour the patients were instructed to come for in-hospital monitoring and evaluation to rule out recurrent preterm labor. The choice of these uterine activity criteria was based on previous studies concerning in-hospital as well as ambulatory tocodynamometry in pregnant patients.[7, 8] After admission to the hospital of a monitored subject, if additional regular uterine activity along with cervical changes could be documented, parenteral tocolysis for recurrent preterm labor was administered. On completion of this treatment, patients were again discharged home to continue home monitoring as before.

Dosage schedules of parenteral and oral tocolytic medications of the two β-sympathomimetics, ritodrine and terbutaline, that were used in this study are described in detail elsewhere.[1-3] Briefly terbutaline (2.5 to 5 mg) or ritodrine (10 to 20 mg) tablets were given every 2 to 6 hours. In group 2 (controls) the fixed dose to be taken at home was the one that controlled contractions during the last 24 hours before discharge, while in the monitored group dosage schedule was adjusted as described above. Patients were instructed to contact their provider if the frequency of contractions was ≥4/hr or if the pulse rate exceeded 110 bpm just before taking the next dose. All patients who were having either objective (monitored) or subjective (control, by palpation) evidence of excessive uterine activity were admitted to the hospital for evaluation of recurrent preterm labor. The in-hospital management of all patients from both groups was identical except for the fact that control patients did not receive a home monitoring device on discharge from the hospital. Oral tocolysis was discontinued at 37 weeks' gestation, and since most practitioners would not initiate parenteral tocolysis after 36 gestational weeks, home monitoring was stopped at that point. The variables relating to pregnancy course and outcome were compared between the groups by the unpaired *t* test and χ^2 analysis where appropriate. The results are expressed as mean ± SD.

Results

As shown in Table II, maternal characteristics were quite similar in both groups. The somewhat insignificant increased mean number of prenatal visits for the monitored group reflects the fact that more of them had longer gestations. Preterm labor was diagnosed at 28.2 ± 2.5 weeks' gestation in group 1 (monitored) patients and at 29.5 ± 2.7 weeks' gestation for group 2 controls. Oral tocolysis for each group was started approximately a week later (group 1, 29.1 ± 3 weeks;

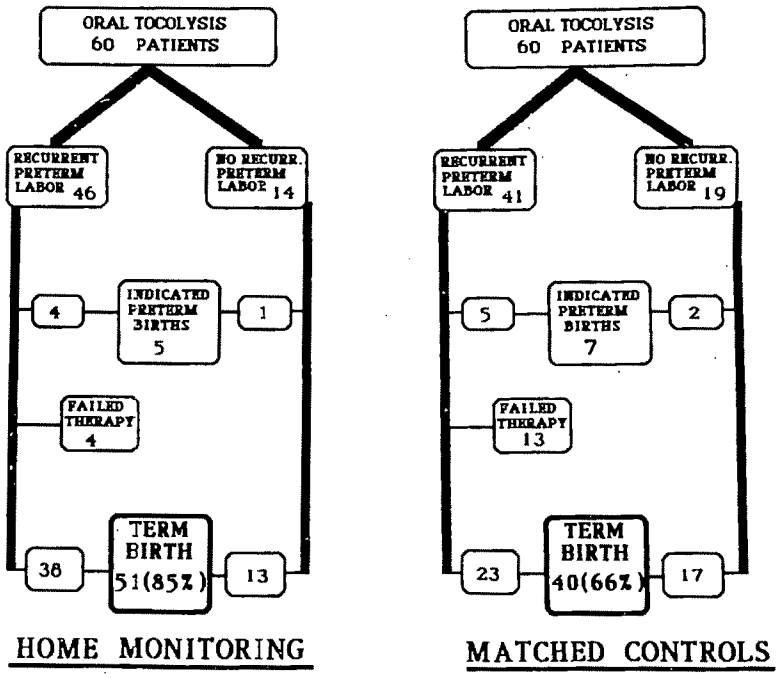


Fig. 1. Pregnancy course and outcome for the study sample.

group 2, 30.7 ± 2.9 weeks), and the mean duration of home monitoring for group 1 patients was 6.4 ± 3.2 weeks. The course and outcome of pregnancies for both groups are illustrated in Fig. 1.

Forty-six (77%) of group 1 (monitored) patients had a total of 83 episodes of recurrent preterm labor (1.8 ± 1.2 episodes per patient) as compared with 41 (67%) of group 2 controls who had 59 episodes of recurrent preterm labor (1.4 ± 1 episodes per patient). Neither the proportion of patients with recurrent preterm labor nor the incidence per patient was significantly different between the groups.

The overall proportion of preterm births was significantly smaller for group 1 (15%) as compared with group 2 controls (34%) ($p < 0.05$). This difference was due to the higher incidence of failed therapy among controls as compared to that of monitored patients (22% versus 7%, $p < 0.05$). The mean time gained in utero from the time that treatment for preterm labor was initiated was significantly longer for group 1 (monitored) patients (7.4 ± 3 weeks) than for group 2 controls (4 ± 1.2 weeks).

Table II. Clinical characteristics of study patients

	Monitored	*Matched*
Age (yr)	26.2 ± 7.5	27.8 ± 7.0
No. of multiparous/No. of primiparous	37/23	35/25
Prenatal care started (wk)	10.0 ± 3.0	9.4 ± 2.7
Bishop score at entry	4.2 ± 2.9	3.9 ± 2.5
Visits/patient (n)	9 ± 3	7 ± 4

Comment

Previous investigators have demonstrated that maintenance of tocolysis by oral administration of β-adrenergic agonists resulted in a significantly decreased incidence of recurrent preterm labor,[3] a significant prolongation of pregnancy,[2] and an improved perinatal outcome.[2] However, unlike other out-of-hospital treatments, such as glucose monitoring of insulin-dependent diabetic women, the management of oral tocolysis could not, up until now, rely on the target organ's response to treatment. In the absence of means to objectively monitor uterine activity during oral tocolysis at home, alternative approaches had to be used. These include prescribing the oral dosage schedule that successfully maintained uterine quiescence during the last 24 hours before discharge,[1] adjustment of treatment to maintain a mild sustained tachycardia 24 hr/day,[1] or reliance on the patient's perception of uterine activity.[5, 6] It seems though that in light of the reported cardiac tachyphylaxis to β-sympathomimetics[9] as well as the relatively limited accuracy of self-perception of uterine activity[10] these approaches are not likely to be successful. Recognizing the need for objective monitoring of uterine activity at home, Zahn,[11] more than a decade ago, was the first to experiment with home ambulatory tocodynamometry for the management of oral tocolytic therapy, but his work was not followed by others.

Determination of the appropriate oral tocolytic maintenance dose while the patient is still in the hospital

may often be inaccurate because of the many unknown factors that are associated with the home environment and may affect uterine activity. It is therefore reasonable to believe that adjustments in dosage schedule may be needed after discharge. In order to carry out these adjustments, preliminary studies to determine the frequency of uterine activity in normal and abnormal pregnancies were performed at an earlier stage.[7] These studies have shown that the criteria for excessive uterine activity that were used in the current study would establish an accurate diagnosis 80% of the time. Therefore the likelihood of overtreatment (placebo effect) in the current study is estimated at 20%. This rate of excessive treatment is well within the range reported by others.[1] On the other hand, the likelihood of significant cervical dilation and effacement when contraction frequency is ≤3/hr is minimal.[7]

An additional potential concern is the adjustment of medications that have the potential for causing adverse cardiovascular side effects.[12] While no clear statement about safety can be made on the basis of the current data, which were obtained from only 120 patients, there are data to show that in the absence of a prolonged tachycardia (≥120 bpm) the likelihood of complications is small.[12] The upper limit of 110 bpm for pulse rate, which was used in this study, seems to be safe.

Since the current study was not controlled by randomization, the question whether the favorable results can be attributed to home tocodynamometry needs to be addressed. Clearly factors such as daily telephone contacts, the availability of an open emergency line, and the posttransmission feedback may all have influenced perinatal outcome. Therefore, while the results for the matched nonmonitored high-risk group compare very favorably with those reported by others,[1-3] it is clear that firm conclusions about the potential role of home monitoring in improving perinatal outcome will need to be confirmed by studies in larger samples and other patient populations.

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The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman

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This retrospective analysis of the use of serum estriol levels for antenatal assessment was performed in an effort to determine if routine, late third-trimester, daily serum estriol monitoring of insulin-dependent pregnant diabetic women can still be justified. Estriol profiles of 170 diabetic pregnancies, managed under a consistent protocol of weekly contraction stress tests and daily serum estriol assessments, were reviewed. A total of 4612 estriol determinations were performed. Nearly 4% of the estriol determinations showed a 35% fall from the mean of the previous three highest consecutive values. Forty-seven percent of the patients had at least one fall of this magnitude. Eighty-five percent of the fetal heart rate tests performed in association with an estriol fall were normal. A fall in estriol was not found to be associated with a higher risk of having a positive contraction stress test, either at the time the estriol fall was recognized or at any time during the patient's antepartum course. Although use of this strict protocol combining the use of weekly contraction stress tests and daily serum estriol determinations provided a safe method of antepartum assessment, there is little evidence to support the routine use of daily serum estriol monitoring in insulin-dependent pregnant diabetic women. (Am J Obstet Gynecol 1986;154:1257-63.)

Key words: Diabetes, estriol monitoring, antepartum assessment

Estriol production by the fetoplacental unit has been used as an index of fetal well-being for a number of years.[1] In the early 1960s, urinary estriol excretion was used to manage pregnancies complicated by diabetes mellitus.[2] Subsequently it became evident that frequent sampling was of great importance if estriol was to be of value as an indicator of fetal status in pregnancies complicated by diabetes. Also evidence was provided that normal estriol patterns indicate an absence of immediate fetal jeopardy.[3-5] Advances in radioimmunoassay techniques, in the mid-1970s, facilitated the routine determination of unconjugated or total plasma estriol levels in many clinical laboratories.[6-8] Clinical experience with both urinary estriol excretion and plasma estriol determinations was associated with improved perinatal outcome in pregnancies complicated by diabetes.[9-11] The need to perform unconjugated plasma estriol determinations on a daily basis with results available within 12 hours, in order to recognize a sudden fall in response to fetal distress with adequate time for successful intervention, was stressed. With the combination of fetal heart rate monitoring, amniotic fluid maturity studies, and daily estriol determinations, it was possible to reduce iatrogenic prematurity while decreasing the perinatal mortality resulting from stillbirths.[10]

Perinatal mortality in pregnancies complicated by diabetes has continued to decline.[12-14] This has been attributed to both better control of maternal diabetes and greater diligence in the use of antepartum fetal surveillance. Increasing importance has been placed on the use of biophysical techniques, including fetal heart rate monitoring and biophysical profile for the management of the pregnant diabetic woman.[13, 15] Estriol monitoring, because of the expense and degree of patient motivation required for its use, has been replaced by these biophysical techniques in many institutions. Because a high degree of success has been achieved in managing pregnancies complicated by diabetes in our institution, we have been reluctant to eliminate the use of estriol monitoring. Therefore we have undertaken this retrospective analysis in an effort to determine if the addition of routine, late third-trimester, daily serum estriol monitoring in insulin-dependent pregnant diabetic patients can still be justified in patients being managed with antepartum fetal heart rate testing.

Methods

Estriol profiles and complete clinical charts were reviewed for 170 insulin-dependent pregnant diabetic women who underwent antepartum fetal surveillance and subsequently were delivered at Women's Hospital, Memorial Hospital Medical Center, Long Beach, between January, 1979, and March, 1984. During this

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Table I. Comparison of patients with at least one fall in estriol with those having no estriol falls

	Estriol fall (n = 79)		*No estriol fall (n = 91)*	
	n	%	*n*	%
Age (yr) (mean)	28.4		29.8	
Parity				
0	41	51.9	38	41.7
≥1	38	48.1	53	58.3
Class				
B	41	51.9	57	62.6
C	15	19.0	19	20.9
D-R	23	29.1	15	16.5
Hypertensive disease*	25	31.6	26	28.6
Incidence of positive CSTs	6/755	0.80	7/662	1.05
Gestational age at delivery				
Mean (wk)	37.2		37.4	
<37 wk	22	27.8	19	20.9
≥37 wk	57	72.2	72	79.1
Intervention at delivery				
None	15	19.0	23	25.3
Elective	37	46.8	43	47.2
Nonelective†	27	34.2	25	27.5
Fetal distress in labor‡	11/28	39.3	12/43	27.9
5-minute APGar score				
≥7	77	97.5	89	97.8
4-6	1	1.3	1	1.1
≥3	1	1.3	1	1.1
Birth weight				
Mean (gm)	3578		3556	
<10th percentile	1	1.3	1	1.1
>4500 gm	9	11.4	11	12.1
Neonatal outcome				
Stillbirth	0		1	1.1
Neonatal death	1	1.3	2	2.2
Discharged alive	78	98.7	88	96.7
Neonatal complications				
Respiratory distress syndrome	5	6.3	7	7.7
Hypoglycemia	8	10.3	15	16.5

*Includes preeclampsia.

†Includes maternal and/or fetal (abnormal CST or estriol) indications for intervention.

‡Includes late decelerations, severe variable decelerations, or prolonged decelerations.

period of time, antepartum assessment in pregnant diabetic patients entailed a consistent protocol of fetal heart rate stress testing and serum estriol levels. Insulin-dependent pregnant diabetic patients were begun on a regimen of weekly contraction stress tests (CSTs) at 34 weeks or earlier as the clinical situation warranted. Testing was begun as early as 26 weeks if intrauterine growth retardation, diabetic nephropathy, or hypertension was present. Antepartum fetal heart rate test interpretation was done by previously described criteria.[16] Biweekly serum estriol determinations were begun at 32 weeks and daily serum estriol measurements were begun at the thirty-fourth week or at the initiation of heart rate testing. Serum unconjugated estriol determinations were performed daily by radioimmunoassay, with the New England Nuclear estriol radioimmunoassay kit.[7] The results were available the same day. A significant fall in estriol was defined as a fall of ≥35% from the mean of the previous three highest consecutive values.[5] A significant fall in estriol elicited the performance of a fetal heart rate test and/or an evaluation of fetal lung maturity with subsequent consideration for delivery. A nonreactive, positive CST usually prompted immediate delivery. Patients with reactive, positive CSTs were delivered if the fetus was mature but were managed with daily estriol measurements and nonstress tests if the fetus was not mature. Equivocal CSTs, defined as suspicious or showing evidence of hyperstimulation, were repeated in 24 hours. Repetitively equivocal CSTs were followed by biweekly nonstress tests and consideration for delivery as soon as maturity could be assured. Patients were not delivered in the absence of documented fetal maturity unless (1) the antepartum fetal heart rate test was nonreactive and positive, (2) the antepartum fetal heart rate test was reactive, positive with abnormal estriol results, or (3) deteriorating maternal condition indicated prompt delivery.

The data for this analysis were taken from chart review and from a prospectively coded, computerized perinatal data base, which included all patients who underwent antepartum fetal heart rate testing and were delivered at Women's Hospital.

Results

There were a total of 4612 estriol determinations performed in the 170 insulin-dependent pregnant diabetic patients, an average of 27 per patient. One hundred seventy-one (3.7%) of the total estriol determinations showed significant falls, which occurred in 79 of the 170 patients (46.5%). A comparison of the 79 patients with at least one estriol fall with the 91 patients with no significant falls revealed no statistical difference with respect to age, parity, class of diabetes, incidence of hypertensive disease, or fetal distress in labor (Table I). Interestingly, although those patients with at least one significant fall in estriol did have more antepartum fetal heart rate tests per patient, there were no more positive CST results in that group than in those patients with no significant estriol falls. There were also no statistical differences in perinatal outcome (gestational age at delivery, indication for intervention, low Apgar scores, mean birth weight, stillbirth, neonatal death, or neonatal complications, Table I)

There was one intrauterine fetal death in this group.

Table II. Patients with significant fall in estriol becoming indication for intervention

APFHRT result	*Previous APFHRT*	*Gestational age (wk)*	*Lecithin/sphingomyelin ratio*	*Maternal risk factors contributing to intervention*	*Neonatal complications*
Positive CST	None	34	ND	—	Neonatal death Renal venous thrombosis
Positive CST	1 day, suspicious CST	35	ND	—	Hypoglycemia
Suspicious NST	1 day, reactive NST	39	1.8 (9 days prior)	Preeclampsia	None
Suspicious CST	1 day, reactive NST	37	4.8	Preeclampsia	Hypoglycemia
Suspicious CST	4 days, negative CST	36	2.1	Preeclampsia	Respiratory distress syndrome
Suspicious CST	2 days, reactive NST	38	2.5	Chronic hypertension	None
Suspicious CST	7 days, negative CST	39	ND	—	None
Hyperstimulated CST	4 days, negative CST	39	1.7 (1 no prior)	—	None
ND	7 days, negative CST	34	2.6	Preeclampsia	None
ND	3 days, negative CST	32	1.9	Chronic hypertension	Respiratory distress syndrome
ND	5 days, negative CST	36	3.1	Preeclampsia	None
ND	3 days, negative CST	35	2.1	Nephropathy	None
ND	2 days, negative CST	36	1.9	—	None
Negative CST	1 day, reactive NST	32	ND	Chronic hypertension Nephropathy	Respiratory distress syndrome

APFHRT, Antepartum fetal heart rate test; ND, not done; NST, nonstress test.

This occurred in a patient at 39 weeks' gestation who refused elective intervention at 38 weeks. A reactive, negative CST was recorded 4 days before the antepartum death, and estriol values were normal until 1 day beforehand when the patient failed to return for daily estriol assessment. There were three neonatal deaths. One occurred in an infant delivered at 34 weeks' gestation without fetal lung maturity testing, after a nonreactive, positive CST, performed for decreased fetal movement, and a 54% fall in estriol level. The autopsy revealed respiratory distress syndrome, renal vein thrombosis, coagulopathy, and intraventricular bleeding. A second neonatal death was attributed to β-streptococcal sepsis, occurring in an infant delivered at 35 weeks with a lecithin/sphingomyelin ratio of 2.1/1. A third neonate, delivered electively at 39 weeks, died on the first day of life and was noted to have bilateral diaphragmatic hernias. Overall, the perinatal mortality rate was 23/1000.

A detailed analysis of the 171 estriol determinations showing a significant fall revealed that for 130 (76%) an antepartum fetal heart rate test was performed on the same day of or the day following the fall in estriol. One hundred ten (84.6%) of these antepartum tests were normal, with 18 (13.8%) being equivocal CSTs and two (1.5%) being positive CSTs.

In 28 falls in estriol (16.4%) the follow-up antepartum fetal heart rate test was performed 2 to 7 days after the significant fall. Twenty-two of these antepartum tests were noted to be normal, while six were equivocal CSTs. There were no positive CSTs noted in this group. The reasons for the delay in performing the test included an inability to contact the patient, patient noncompliance in returning for the tests, and delayed recognition of the estriol fall. An abnormal test, performed after a delay, prompted delivery in only one instance. This occurred in a Class F diabetic patient at 38 weeks, in whom a suspicious CST performed 5 days after a fall in estriol prompted an evaluation of fetal lung maturity and subsequent delivery. The estriol values in the interim were normal. No stillbirths or otherwise adverse outcomes were noted in this group of patients in whom performance of the antepartum test was delayed.

No antepartum fetal heart rate test was performed before delivery in 13 cases (7.6%). These patients were delivered without testing for the following reasons: The estriol fall led to an evaluation of fetal lung maturity and subsequent intervention (n = 5); previously scheduled elective induction of labor or cesarean section was performed (n = 2 each); spontaneous labor ensued (n = 3); or the estriol fall was unrecognized (n = 1).

Fourteen patients (8.3%) had falling estriol values as an indication for intervention (Table II). Only two patients had associated positive CSTs, and another six patients had associated equivocal CSTs. In only one of these patients with abnormal tests was the test performed before scheduled testing because of the finding of an estriol fall. Patients being tested at intervals of <7 days had additional indications or maternal risk factors necessitating more frequent testing. There was

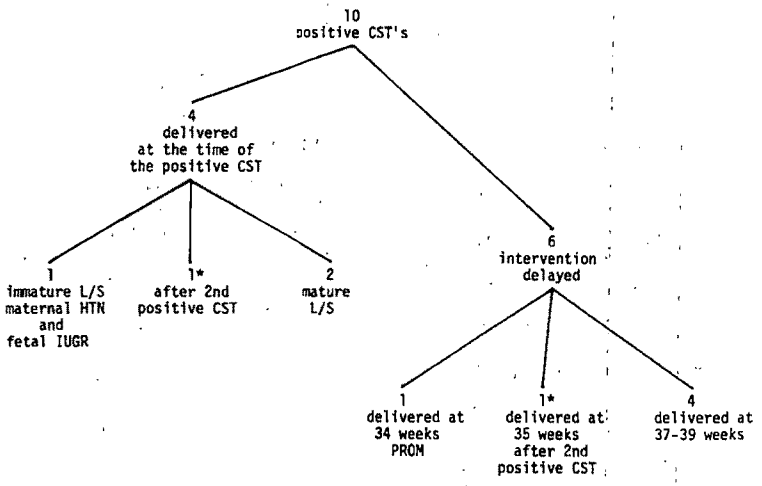


Fig. 1. Pregnancy outcome for nine patients with 10 positive CSTs and normal estriol values. *L/S,* Lecithin/spingomyelin ratio; *HTN,* Hypertension; *IUGR,* intrauterine growth retardation; *PROM,* premature rupture of membranes. * = Same patient.

Table III. Positive CSTs

Gestational age at time of positive test (wk)	*Same-day estriol result*	*Gestational age at time of delivery (wk)*	*Intervention at time of positive test*	*Neonatal complications*
31*	Normal	34	Delayed	None
34	Normal	39	Delayed	None
34	Normal	34	Delivered, lecithin/sphingomyelin ratio 1.2	Respiratory distress syndrome
34	Normal	34	Delivered, lecithin/sphingomyelin ratio 2.7	Hypoglycemia
34	Normal	37	Delayed	Hypoglycemia
34†	Normal	35	Delayed	None
35†	Normal	35	Delivered, no lecithin/sphingomyelin ratio	None
35	Normal	38	Delayed	None
36	Normal	36	Delivered, lecithin/sphingomyelin ratio 4.2	None
36	Normal	37	Delayed	None
34	54% Fall	34	Delivered, no lecithin/sphingomyelin ratio	Neonatal death Renal venous thrombosis
35	60% Fall	35	Delivered, no lecithin/sphingomyelin ratio	Hypoglycemia
29*	Not done	34	Delayed	None

*Same patient.
†Same patient.

no demonstrable morbidity associated with intervention secondary to an estriol fall.

A review of all antepartum fetal heart rate tests performed revealed 11 patients who had 13 positive CSTs. These tests were evenly distributed between the 79 patients with at least one fall in estriol and the 91 patients with no estriol falls. Of patients with at least one fall in estriol, 6.3% had a positive CST, compared with 6.6% of patients with no estriol falls. Gestational age at the time of the positive test ranged between 29 and 36 weeks (Table III). Estriol values were normal at the time of 10 positive CSTs in nine patients (Fig. 1). Intervention was delayed in six instances of positive CSTs because of normal estriol values. These six positive CSTs were reactive. The infants were subsequently delivered at 34 to 39 weeks, and all had benign neonatal courses.

A review of all the estriol profiles included an assessment of the number of significant falls that were ≥40% compared with those that fell between 35% and 40% (Table IV). A fall in estriol ≥40% was associated with no more abnormal antepartum fetal heart rate tests than a fall between 35% and 40%. In addition, the only two positive CSTs associated with a fall in estriol were associated with a fall >40%. If 40% had been used to define a significant estriol fall, there would have been

a decrease in estriol falls of 37% and 28% fewer patients would have had an estriol fall, without a failure to detect those falls associated with positive CSTs.

Comment

The incidence of intervention in diabetic pregnancies is necessarily high in an effort to avoid stillbirth.[9, 12] Only 22% of these 170 insulin-dependent diabetic patients entered spontaneous labor. Intervention, however, does carry with it the risk of complications secondary to prematurity. Therefore the timing of intervention is extremely important and complex. It is evident that a number of factors come into play in deciding when to intervene in a diabetic pregnancy. Gestational age, fetal lung maturity, maternal condition, antepartum fetal heart rate test results, and estriol profiles were all important factors leading to intervention in this group of patients. In only rare instances was a single factor the sole indication for intervention. A significant fall in estriol was a factor in the decision to intervene in 8% of patients in this study group. An estriol fall was the sole indication for intervention in only a single instance. Only one estriol fall (0.6%) was the initial inciting event that triggered further evaluation and subsequent intervention. Although a number of factors proved to be important in the decision to intervene, this study did not confirm the importance of using more than one modality of antepartum fetal surveillance, as suggested in previous studies of pregnant diabetic women.[11-13]

Estriol determinations, in addition to being a factor leading to intervention, were also used to prevent unnecessary premature intervention. Six patients (3.5%) between 31 and 36 weeks' gestation were allowed to continue the pregnancy despite reactive positive CSTs in the face of normal estriol profiles. Further assurance that these pregnancies did not require intervention was provided by the presence of reactivity on each of the positive CSTs. Daily nonstress tests evaluating reactivity may well have allowed a similar delay of intervention.

Estriol monitoring in pregnant diabetic patients requires daily testing with prompt evaluation of patients having significant falls.[5, 10] The antepartum fetal heart rate tests performed in association with estriol falls were normal 85% of the time. Only 1.5% of antepartum fetal heart rate tests performed at the time of a fall in estriol were positive CSTs. The incidence of positive CSTs among antepartum fetal heart rate tests performed in the absence of an estriol fall was 0.85%. This difference was not statistically different. The incidence of positive CSTs and the percent of patients having positive CSTs were not different when patients with at least one estriol fall were compared with patients with no estriol falls. Thus a fall in estriol was not associated with a higher risk of having a positive CST, either at the time the fall was recognized or at any time during the patient's antepartum course.

Table IV. Comparison of percent fall in estriol with incidence of abnormal antepartum fetal heart rate test

% Fall	*No. of estriol determinations*	*% Patients with at least 1 fall*	*% Associated with abnormal APFHRT*
≥40	107 (2.3%)	33.1	14.0*
35-40	64		17.2
≥35	171 (3.6%)	46.5	15.2

Numbers in parentheses are percentages of total estriol determinations performed; APFHRT, antepartum fetal heart rate test.

*The only two positive CSTs associated with a fall in estriol had a fall >40%.

It has been unclear what level of sensitivity is necessary to detect fetal jeopardy at all times and minimize the number of falsely positive values associated with the use of estriol monitoring. Because of the relatively low incidence of positive CSTs in a well-controlled diabetic population, it has been difficult to establish what percent fall in estriol should elicit further evaluation. The data from this study, as well as others, suggest that a 40% fall from the mean of the previous three highest consecutive values is adequately sensitive.[11, 12] The use of a 40% fall limit in this study group would have decreased the number of abnormal estriol values by 37% and decreased the number of patients with at least one significant fall by 28%, without failing to detect those patients with positive CSTs.

In conclusion, the current role of daily serum estriol determinations in the management of the insulin-dependent pregnant diabetic patient can be summarized as follows:

1. Estriol results may provide information to help determine timing of intervention; however, rarely if ever will estriol results alone prompt intervention or initiate evaluation that leads to intervention.

2. Daily estriol results showing normal values may allow delay of intervention in patients <37 weeks' gestation with positive CSTs; however, daily nonstress tests evaluating reactivity may provide the same assurance that intervention can be delayed.

3. Routine daily estriol screening is expensive and time-consuming for the patient and adds little or nothing to the detection of fetal jeopardy when used in conjunction with fetal heart rate stress testing.

4. If daily estriol monitoring is to be used in certain circumstances, a 40% fall limit is an adequately sensitive cutoff for further evaluation.

Although this strict protocol combining the use of weekly CSTs and daily serum estriol determinations

provided a safe method of antepartum assessment, we cannot justify the continued routine use of daily serum estriol monitoring in insulin-dependent pregnant diabetic patients. Other methods of fetal surveillance that are less time-consuming and cumbersome, such as midweek nonstress tests or fetal movement counting, may prove to be effective adjuncts to weekly CSTs in pregnancies complicated by insulin-dependent diabetes. Investigation in these areas is currently in progress.

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Discussion

DR. ROBERT RESNIK, San Diego, California. Dr. Freeman and his colleagues have reported the pregnancy outcomes of 170 gravid women with type I diabetes mellitus, with respect to the comparative reliability and sensitivity of antepartum fetal heart rate monitoring and daily serum estriol testing. It has been the policy at their institution for several years to use these dual techniques to evaluate fetal well-being in the diabetic patient.

Data from this type of retrospective study can be difficult to interpret. This is due to the fact that management protocols that are not prospective in nature allow more for clinical flexibility than for critical scientific investigation.

Nonetheless, it seems clear from Dr. Freeman's data that antepartum fetal heart rate monitoring is a satisfactory technique for monitoring fetal well-being and that serum estriol testing, and I quote from the text, "adds little or nothing to detection of fetal jeopardy when used in conjunction with fetal heart rate testing."

This is not surprising when one considers some of the biochemical aspects of estriol synthesis. The rationale for its use in monitoring high-risk pregnancies is based on the production of enormous amounts of estrogenic precursors by the fetal adrenal gland, which are then converted to C-18 steroids by the placenta. The placental mass apparently has a large reserve in enzymatic capability for aromatization of ring A or the final conversion of 16α-hydroxylated DHEA to estriol. Consequently, only sharp decreases in fetal precursor production or profound placental dysfunction will result in decreased total estriol production. The clinical significance of these biochemical realities is that a fetus or its placenta must be in serious jeopardy before the clinical status will be reflected by a fall in maternal serum estriol.

Despite the lack of well-controlled clinical studies in the past, a serum estriol value in the normal range or increasing values have generally been reassuring to clinicians. However, a decreasing estriol value has been misleading with respect to fetal status more often than not. This is borne out by the finding in this study that 46% of the patient population had at least one significant drop in estriol and there was only one fetal death in the entire group. Furthermore, in only one instance was a fall in estriol the primary event that ultimately triggered obstetric intervention.

This study confirms the very limited value of estriol in monitoring the diabetic pregnancy and reinforces the use of antepartum fetal heart rate monitoring for high-risk pregnancies in general. I would ask Dr. Freeman the following question: At the outset of the study, it is stated that estriol levels would not be used in decision making regarding delivery timing unless the CST was interpreted as being positive but reactive, and the fetal lung was immature. If so, how many of the 170 patients met those specific criteria, and if the number

is small, as I would expect it would be, can you justify the expense of an estriol laboratory?

Dr. James Caillouette, Pasadena, California. I would like to add one more nail to the estriol coffin. I thank Dr. Freeman for providing me the opportunity to do so.

In 1970, I was the formal discussant for Dr. Roger Hoag's paper on the use of estriol testing for the evaluation of fetal well-being. I was skeptical at that time and registered significant reservations.

During the past 15 years many articles have been written on the subject of estriol testing for evaluation of fetal well-being. Most present either mixed or negative data.

Both Dr. Freeman and Dr. Resnik have made clear that this test should at long last be given a decent burial.

Dr. Freeman (Closing). Dr. Resnik, it is ironic that you were one of the first to point out your skepticism about the value of estriol monitoring for fetal well-being and now you are discussing this article. You now have some data to support your bias and I want to compliment you on your previous clairvoyance.

Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization

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San Diego, California

Sixty-nine patients with tubal infertility secondary to pelvic inflammatory disease were surgically treated by one of three infertility surgeons, who used microsurgery for repair of the tubal pathologic condition and early second-look laparoscopy 6 to 30 days postoperatively for lysis of postoperative adhesions. No patient was included in this group whose disease was thought to have originated from endometriosis or prior abdominal surgery. The average follow-up time was 43.1 months (range 12 to 85.9). Nine patients were excluded from the analysis. Pregnancy outcome by procedure, expressed as the percentage of patients conceiving, was as follows: adhesiolysis, 69% (61% term, 8% ectopic); fimbrioplasty, 35% (25% term, 10% ectopic); salpingostomy, 30% (18% term, 12% ectopic); and cornual implantation, 60% (40% term, 20% ectopic). No added therapeutic value could be attributed to the use of early second-look laparoscopy. Given the relatively poor outcome of fimbrioplasty and salpingostomy, it may be prudent to advise patients with bilateral partial and/or total tubal occlusion against tuboplasty in favor of in vitro fertilization and embryo transfer. (AM J OBSTET GYNECOL 1986;154:1264-70.)

Key words: Tubal infertility, microsurgical treatment, laparoscopy, second look, inflammatory disease, in vitro fertilization

Approximately 13% of women who experience a single episode of pelvic inflammatory disease will suffer infertility secondary to postinfectious tubal occlusive disease.[1] In the United States during the late 1970s, 10 to 13 cases of pelvic inflammatory disease per thousand women aged 13 to 34 years were reported annually.[2] Westrom[3] calculates that this incidence corresponds to approximately 500,000 new cases of postinfectious tubal infertility per year. Often these women are nulliparous and are unaware of having had an episode of pelvic inflammatory disease.[4] Tubal corrective surgery has been the main approach to the treatment of this cause of infertility.[5] Microsurgical technique has been shown to improve the results of such surgery in some studies[5-8] but not in others.[9, 10] This article (1) examines the results of tubal microsurgery for infertility when the surgery is followed by early second-look laparoscopy 6 to 30 days postoperatively to lyse postoperative adhesions in patients suffering from postinflammatory tubal adhesive disease and (2) considers the implications of these results in deciding in the future which patients with tubal-factor infertility would be best served by microsurgery and which by in vitro fertilization and embryo transfer.

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Material and methods

Study group. The study group originally consisted of 69 women who came to a private fertility clinic with a chief complaint of infertility (defined as a failure to conceive after 1 year of adequate exposure), whose infertility was determined, after study, to be due to postinflammatory tubal disease, and who underwent microsurgery and early second-look laparoscopy between April, 1978, and June, 1983. The average age of the patients at the time of surgery was 28.3 years (range 22.5 to 39.5). The patients came from a broad spectrum of socioeconomic and racial backgrounds. Of the 69 patients, three had undergone previous surgery for the correction of infertility. Nine patients were excluded from the analysis for the following reasons: four had less than 1 year of follow-up; four had less than 1 year of sexual exposure after the surgery; and one was found to have one normal tube at the time of surgery that required no correction. Thus the final group available for analysis consisted of 60 patients.

Evaluation. All patients in this group underwent an infertility evaluation prior to surgery, which included basal body temperature charts, postcoital test, semen analysis of the male partner, demonstration of ovulation (usually with a midluteal serum progesterone de-

Table I. Postoperative pregnancy rates, by procedure, for this study*

Procedure	No. of patients	Outcome of first pregnancy				Eventual outcome	
		%IUP	%SAB	%Term	%Ectopic pregnancies	%EIUP	%E term
Adhesiolysis	13	62 (8)	8 (1)	54 (7)	8 (1)	69 (9)†	62 (8)
Fimbrioplasty	20	30 (6)	15 (3)	15 (3)	10 (2)	35 (7)	25 (5)
Salpingostomy	17	12 (2)	0	12 (2)	12 (2)	18 (3)	18 (3)
Cornual resection	10	30 (3)	0	30 (3)	20 (2)	50 (5)‡	40 (4)
Total	60	32 (19)	7 (4)	25 (15)	12 (7)	38 (24)	33 (20)

IUP = Intrauterine pregnancy; SAB = spontaneous abortion; EIUP = eventual intrauterine pregnancy; E term = eventual term pregnancy.

*Numerals in parentheses represent number of patients.

†Adhesiolysis versus fimbrioplasty significant at 0.05; adhesiolysis versus salpingostomy significant at 0.01.

‡Cornual resection versus salpingostomy significant at 0.05. Other pairings do not achieve statistical significance.

Table II. Results of adhesiolysis: Representative literature reports

	No. of patients	%Live births	%Ectopic pregnancies	?ESLL
Conventional technique				
Wallach et al.[10] (1983)	94	45.7	2.1	No
Young et al.[14] (1970)	47	32.0	4.0	No
Trimbos-Kemper et al.[15] (1985)	N/A	48.0	N/A	Yes
Microsurgical technique				
Frantzen and Schlösser[9] (1982)	49	38.8	4.1	No
Diamond[6] (1979)	140	57.1	0.7	No
Luber et al. (1985)	13	62.0	7.7	Yes
Hulka[12] (1982)	47	25.5	2.1	No
Laparoscopic lysis only				
Gomel[5] (1983)	92	58.7	5.4	—

ESLL = Early second-look laparoscopy; NA = not available.

termination), hysterosalpingography, and preoperative laparascopy with chromopertubation.

Procedures. The operative procedures were performed by one of the three infertility surgeons participating in this study at one of three hospitals in the San Diego area. The three surgeons are partners in private practice, regularly assist each other during surgery, and have thus evolved a common technique. Most of the surgeries were performed at University of California, San Diego, Medical Center. Reconstruction of the fallopian tubes and lysis of pelvic adhesions were performed with the use of microsurgical techniques, including minimal tissue trauma, meticulous hemostasis by means of fine electrocautery, reperitonealization of all denuded surfaces, prevention of tissue desiccation with continuous irrigation, and 10× to 16× magnification with an operating microscope. Vicryl suture (8-0) was used on the fallopian tubes. All patients received antibiotics and steroids before, during, and after operation. Thirteen patients underwent temporary suspension of the ovaries to the round ligament or uterine fundus with a 1-0 Prolene suture in addition to their primary tubal procedure. These suspensions were removed at the time of second-look laparoscopy. Immediately before the incision was closed, 200 ml of Hyskon or, in some early cases, 500 ml of 32% dextran 70 was instilled into the peritoneal cavity.

Early second-look laparoscopy was performed on all but one patient between 6 and 30 days postoperatively, the mean interval being 11.1 days. At the time of early second-look laparoscopy, the surgical outcome was evaluated for tubal patency and extent of adhesions. Usually the adhesions found at early second-look laparoscopy were able to be cleared by means of a blunt probe inserted suprapubicly. Ovarian suspensions, when present, were released, and phimotic tubal ostia were opened when necessary. On conclusion of the procedure, 200 ml of Hyskon or 500 ml of 32% dextran 70 was instilled in the peritoneal cavity through the laparoscopic trocar sleeve.

Classification of cases. Cases were classified as adhesiolysis, fimbrioplasty, salpingostomy, or cornual resection. As recommended by the International Fertility Society,[5] fimbrioplasty was separated from salpingostomy by the preoperative presence of a tubal lumen. When a patient required a different procedure on each adnexum, the patient was classified according to the adnexum of lesser pathology. This classification

Table III. Results of fimbrioplasty: Representative literature reports

	No. of patients	*%Live births*	*%Ectopic pregnancies*	*?ESLL*
Conventional technique				
Wallach et al.[10] (1983)	24	20.8	8.1	No
Young et al.[14] (1970)	24	25.0	8.1	No
Trimbos-Kemper et al.[15] (1985)	33	51.0	N/A	No
Trimbos-Kemper et al.[15] (1985)	52	45.0	N/A	Yes
Microsurgical technique				
Frantzen and Schlösser[9] (1982)	49	22.4	4.1	No
Patton[7] (1982)	40	63.0	5.0	No
Luber et al. (1985)	21	25.0	9.5	Yes

ESLL = Early second-look laparoscopy; N/A = not available.

Table IV. Results of salpingostomy: Representative literature reports

	No. of patients	*%Live births*	*%Ectopic pregnancies*	*?ESLL*
Conventional technique				
Rock et al.[11] (1978)	99	16.0	6	No
Young et al.[14] (1970)	18	16.7	0	No
Trimbos-Kemper et al.[15] (1985)	68	24.0	17	No
Trimbos-Kemper et al.[15] (1985)	95	19.0	10	Yes
Microsurgical technique				
Frantzen and Schlösser[9] (1982)	85	14.1	3.5	No
Gomel[5] (1983)	89	31.5	9.0	No
Hulka[12] (1982)	61	9.8	8.1	No
Luber et al. (1985)	18	18.0	12.0	Yes

ESLL = Early second-look laparoscopy;

method was used (1) because it is assumed that if these patients become pregnant, it will be by way of the less affected tube and (2) because the classification is in keeping with the recommendatons for standardization put forth by other authors.[5, 9] Patients who required cornual resection of either tube, with or without additional surgery, were separately analyzed.

It is evident that since the patient's disease state at the time of exploratory laparotomy dictates the type of surgery performed, classification of patients by type of surgery performed is also a classification of the extent of disease.

Results

The outcome of these procedures is summarized in Table I. Intrauterine pregnancy rates are broken down into term pregnancy rates and spontaneous abortion rates. Ectopic gestations are listed separately. In addition, we have included the category of "eventual intrauterine pregnancy rate," which includes patients who eventually had an intrauterine pregnancy but only after a postoperative ectopic pregnancy, and the category of "eventual term pregnancy rate," which includes patients who eventually delivered a viable fetus but only after a postoperative abortion or an ectopic pregnancy.

For the 60 patients included in our analysis, the average follow-up time was 43.1 months (range 12 to 85.9). The intrauterine pregnancy rate varied inversely, and the ectopic rate varied directly, according to the extent of disease encountered and the complexity of the procedure required. Adhesiolysis resulted in the highest term delivery rate (62%) and the lowest incidence of ectopic gestations (8%). Salpingostomy produced the lowest term delivery rate (18%) and the highest ectopic rate (12%). Fimbrioplasty resulted in intermediate values of term delivery (25%) and ectopic gestation (10%). The differences were statistically significant for adhesiolysis versus both fimbrioplasty and salpingostomy (Table I). Any conclusions regarding the cornual resection group are limited by its size, especially since within this small group there are three subsets (two patients had cornual reanastomoses after resection, two patients had "mixed" procedures [cornual resections combined with distal tubal surgery], and six patients had cornual reimplantation after resection). Nevertheless, a high rate of both term gestations (40%) and ectopic gestations (20%) was noted within this entire group, and the intrauterine pregnancy rate for this

Table V. Results of IVF: Fertility Institute, 1985

	%A	%B	%C	%D	%E	%F
A. No. of stimulated cycles: 28						
B. No. of laparoscopies: 19	68					
C. No. of laparoscopies with one or more eggs retrieved: 18	64	95				
D. No. of laparoscopies resulting in one or more fertilized eggs: 14	50	74	78			
E. No. of transfer events*: 14	50	74	78	100		
F. No. of clinical pregnancies:† 4	14	21	22	29	29	
G. No. of continuing pregnancies: 4	14	21	22	29	29	100

*One or more fertilized eggs transferred.

†Excludes "chemical" pregnancies. Requires either rising human chorionic gonadotropin levels with one value >2000 IU or intrauterine sac on sonogram to be included here.

group, versus the salpingostomy rate, was statistically significant (Table I).

The percentages of patients who achieved two or more pregnancies postoperatively were as follows: adhesiolysis, 32%; fimbrioplasty, 5%; salpingostomy, 12%; and cornual resection, 20%. Since many of our patients chose to have only one pregnancy after surgery, these data do not represent the total capacity for multiparity after surgery.

There was no statistically significant difference in the time interval from procedure to conception among the four surgical groups. The shortest average time was 10.9 months (fimbrioplasty), and the longest was 15.3 months (adhesiolysis). The average time interval from procedure to term pregnancy for the entire group of 60 patients was 13.7 months, whereas the average time interval from procedure to ectopic pregnancy was 9.1 months.

When the pregnant and nonpregnant groups were analyzed, no statistically significant differences were found regarding the ages of the patients (28 versus 28.7 years), a history of previous corrective or abdominal surgery, the time from operation to early second-look laparoscopy, or the follow-up time. Ovarian suspension was generally performed for the most severe adhesive states. Thirteen patients underwent ovarian suspension as part of their procedures, but distribution of this surgical modification among the four procedures occurred in such a way that no conclusion could be drawn as to its efficacy. There were six ovarian suspensions in the pregnant group and seven in the nonpregnant group.

The time interval from operation to early second-look laparoscopy did not statistically differ for the pregnant versus the nonpregnant groups, for the ectopic versus the term groups, or for each of the four procedure groups.

At early second-look laparoscopy, adhesions were graded on a scale from 0 to 3 where 0 equaled no adhesion, 1 equaled filmy adhesions, 2 equaled moderate adhesions, and 3 equaled dense, difficult-to-manage adhesions. When patients were placed into two groups having grade 0 to grade 1 adhesions in one group and grade 2 to grade 3 adhesions in the other group, there was no statistically significant difference demonstrable in the probability of having either an ectopic or a term gestation, in the probability of becoming pregnant, or, for those patients in the pregnant group, in the time interval between operation and conception.

Comment

It has been repeatedly demonstrated[9, 11-14] that the intrauterine pregnancy outcome after tubal surgery is inversely proportional, and the ectopic pregnancy outcome directly proportional, to the extent of the patient's original disease. This finding has become virtually an axiom in tubal surgery. Our data confirm these observations. Tables II to IV suggest that, in the literature, this relationship holds true whether conventional or microsurgical techniques are used and whether or not early second-look laparoscopy is added to the operative procedure. These tables incidentally also tend to support the view, with which we concur, that there is no clear-cut advantage to microsurgery with or without early second-look laparoscopy over conventional surgery for the treatment of any of the disease categories.

The inverse relationship described above between degree of tubal disease and pregnancy outcome after surgical correction of tubal infertility does not pertain to in vitro fertilization with embryo transfer, in which the intrauterine pregnancy outcome is independent of the degree of tubal pathology,[16] provided that the ovaries are accessible for oocyte retrieval. On the basis of this important difference between surgery and in vitro fertilization with embryo transfer, Wood and Trounson[16] have suggested that "in vitro fertilization and embryo transfer may be preferred to tubal surgery where [the expected success rate of the surgery is] less than the cumulative pregnancy rate of three laparoscopies for [in vitro fertilization] (about 40%)."

Table V shows that data for our community hospital–based in vitro fertilization with embryo transfer

program for the calendar year 1985 to August 30, 1985. Probably the most useful way of reporting in vitro fertilization with embryo transfer pregnancy outcomes, from the patient's point of view, is the pregnancy rate per laparoscopy. In our hands over this period, one in vitro fertilization with embryo transfer laparoscopy had an intrauterine pregnancy rate of 21% and an ectopic rate of 0%, regardless of the extent of tubal pathology. These data are similar to those reported by others[16] and, as reported in this paper, compare with postsurgical intrauterine pregnancy rates of 61%, 25%, and 18% following adhesiolysis, fimbrioplasty, and salpingostomy, respectively, and with corresponding ectopic rates of 8%, 10%, and 12%. It is important to realize that this comparison is between patient groups handled by the same three physicians for each group and therefore represents truly comparable results.

By way of further comparison, one complete in vitro fertilization with embryo transfer cycle costs less than half as much as a complete microsurgery–early second-look laparoscopy cycle and results in dramatically less discomfort, risk, and postoperative disability. It has the additional advantage that the pregnancy outcome is known 30 days after the cycle has begun, whereas the waiting time for pregnancy after surgery may be up to 4 years.

These considerations suggest to us that, in our hands, most cases of tubal infertility amenable to adhesiolysis would be best treated with the surgical approach. However, in those cases in which it is believed preoperatively, on the basis of hysterosalpingography and/or laparoscopy, that fimbrioplasty or salpingostomy would be required in the surgical treatment of the tubal pathology, in vitro fertilization will in most cases be the preferred modality of treatment. This conclusion is in agreement with the conclusions reached by others.[13, 16]

We conclude that microadhesiolysis with early second-look laparoscopy is a highly successful surgical procedure (61% intrauterine pregnancy rate) but that microfimbrioplasty with early second-look laparoscopy (25% intrauterine pregnancy rate) and microsalpingostomy with early second-look laparoscopy (18% intrauterine pregnancy rate) are much less so. In our hands, patients requiring either of these latter two procedures appear in most cases to be better advised to attempt in vitro fertilization with embryo transfer (21% intrauterine pregnancy rate per laparoscopy event), since even a single in vitro fertilization laparoscopy has an intrauterine pregnancy rate similar to that of microfimbrioplasty and/or microsalpingostomy but is associated with less discomfort, disability, risk of ectopic pregnancy, and expense for the patient.

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Discussion

Dr. Richard M. Soderstrom, Seattle, Washington. With the increase of pelvic inflammatory disease and the marked decrease in babies available for adoption, it's no wonder that microsurgery has received such ferocious attention from reproductive surgeons. Despite many sophisticated improvements in microsurgical techniques and instruments, viable pregnancy in most circumstances follows such surgery in less than half the patients subjected to this major surgical event. Follow-up laparoscopy has been recommended by most surgeons; some recommend it within a few weeks of microsurgery, whereas others recommend a 4- to 6-month delay. In this flurry of infertility surgery, the patients and their physicians have watched with interest the glamour and advancements of in vitro fertilization and embyro transfer. Although the chemical pregnancy rate in most seasoned centers is impressive, the number of live births has been disappointing Nevertheless, improvement is reported with each year.

Dr. Young and his associates have analyzed 60 patients who had damaged fallopian tubes following one or more attacks of pelvic inflammatory disease and have searched their experience for success and failure. They

have considered ectopic pregnancy as a failure, and I'm sure their patients agree. They have separated this group of 60 patients into four categories according to procedure, hoping to answer two questions that I interpret as being the following. First, when should the surgeons recommend in vitro fertilization? Second, should they recommend microsurgery instead? In doing so, Dr. Young and associates were unable to demonstrate a startling improvement following early second-look laparoscopy when their results were compared with those reported in the current literature.

However, this study does not compare *late* second-look laparoscopy and the resultant pregnancy rate, which is still favored by many surgeons. Perhaps the lysis of freshly formed adhesions promotes re-formation of the same adhesions. Personally, I've been impressed with the paucity of adhesion re-formation after laparoscopic lysis of old adhesions has been performed for other indications.

Unfortunately the confidence limits are too broad, and thus the power of this study is too weak to answer those two questions. With such a small series sprinkled with confounding variables, such as the extent of adhesions in each subgroup, a remarkable difference in outcome would have to be evident before any cause-and-effect relationship could be entertained. Diseased tubes do not heal well under the best conditions or at the hands of the best surgeon. To classify the extent of the adhesions is helpful, but the other variables, such as epithelial integrity of the tube, will soften the analysis.

All of the patients received preoperative laparoscopy, and in the article there is a quote from the literature regarding adhesiolysis in three different modes: (1) the conventional technique, in which live-birth rates ranged from 48% down to 32%, (2) microsurgical techniques, in which rates were as low as 25% and as high as 62%, and (3) creative laparoscopy, as reported by Gomel, who had 92 cases with a live-birth rate of 58.7%. In Gomel's series, however, all the adhesions were handled by operative laparoscopy, not by microsurgery, and I wish to ask Dr. Young whether any of the patients in his practice underwent laparoscopic lysis of adhesions, rather than microsurgery, or did all of them come to microsurgery? I believe that if the latter was the case, this would certainly influence the statistics.

I was somewhat startled by the cost comparison of an in vitro fertilization in San Diego versus microsurgery. This is not the case in Seattle, since microsurgery is only about 20% higher than one course of laparoscopy for vitro fertilization.

Although the inverse relationship between pregnancy outcome and the degree of tubal disease does not pertain to in vitro fertilization, one cannot assume that all those patients who would have conceived after microsurgery will conceive after in vitro fertilization. Let us assume that the statistics from abroad and from Australia give a 20% expected live-birth rate following one laparoscopy. Dr. Young implies in his presentation that these statistics would hold up with subsequent laparoscopies, at least after four laparoscopies with in vitro fertilization. It would mean that after one laparoscopy in 100 patients, there would be 20 live births. After two laparoscopies, there would be 16 more; after three there would be 13 more; and after four there would be 10 more. He therefore predicts 59 pregnancies in 100 patients who would elect four laparoscopic retrievals for in vitro fertilization procedures—an ambitious statistic that has yet to be proved. If the 20 patients who would have become pregnant following fimbrioplasty or salpingostomy had instead chosen in vitro fertilization and embryo transfer, the same statistics would apply. Thus eight of those 20 patients would not conceive.

We are all here today because we are certain of our impressions but frequently skeptical of facts. Dr. Young and his associates have reminded all of us, with this analysis, that the benefit-risk ratio of microsurgery for infertility must receive periodic reappraisal and be compared with the ratio for newer physiologic and technologic advances in the area of reproductive health.

Dr. Kelvin Churches, Carlton, Australia. I'm hesitant to speak, because I have no firm figures from the in vitro fertilization units, but I think that when you talk about the leading in vitro fertilization units in Australia, both come from Melbourne, one at my hospital, the Royal Women's Hospital, and one at Queen Victoria Hospital. I must be less optimistic than Dr. Young about the projections, and I agree with Dr. Soderstrom in that, to the best of my knowledge of the patients in the in vitro fertilization programs, each of which have up to three laparoscopies, the current yearly rate of successful pregnancy varies from 12% to 20%, and it hasn't gone above 20%.

Dr. Simon Henderson, Los Angeles, California. Thank you, Dr. Young, for that good presentation. However, I endorse what Dr. Soderstrom said, namely, that there is no real proof that early laparoscopy is better than late laparoscopy or than laparoscopy done only if the patient doesn't get pregnant. In addition, I strongly suggest caution about term pregnancy results with in vitro fertilization. In fact, I think all of us who have read the article in *Fertility and Sterility* by Dr. Soules—the title of which is "IVF results: Let's be honest"—will be aware of the somewhat flamboyant pregnancy rates reported in the press. I think that caution is therefore appropriate. I also agree with Dr. Churches' comments that probably, even in the best clinics, we're not getting much more than a 20%, and maybe in some months a 30%, ongoing pregnancy rate with in vitro fertilization.

I also wish to comment on the number of microsurgical cases you are doing. By my calculations the rate is probably about one procedure a month over the 6 years, coming up with your total of 69 cases. In my practice, I find that microsurgical operations for patients with pelvic inflammatory disease represent about half of my microsurgery; 25% are reversals of sterilization, and the last 25% are for endometriosis. Thus I estimate that you probably do two microsurgical tubal procedures a month. I forget how many partners you said you have, but I think it was two or three, which

means only a few microsurgical cases a month for each of you. Most microsurgeons believe that they should be doing about two or three cases a week to be keeping their hand in, so to speak. In addition, your results with the salpingostomies for the hydrosalpinx, the very bad cases, were a bit low, that is, 18%. These results are low in comparison with Winston's and Gomel's results (25% to 35% term pregnancy rates), which are similar to what I find in my office, and may reflect the relatively few microsurgical procedures you are doing.

Dr. Donald Minkler, San Francisco, California. Dr. Young, your presentation has raised some valuable and interesting questions that might have applicability in other parts of the world. I have had an opportunity to observe very high levels of infertility in developing countries, particularly in Africa, because of the tubal factor caused by untreated sexually transmitted disease. In most institutions in these areas, surgical treatment is simply not feasible because it's too costly and because the demands on very limited resources for surgery are too high to allow for elective procedures such as tuboplasty. At the same time the social consequences of infertility for the woman are very profound. Therefore, I want to ask whether you think that the cost factor and the level of laboratory sophistication that is required for in vitro fertilization have reached, or will soon reach, a point where it is realistic for us to begin to think about this procedure as a practical alternative to surgical management in less developed countries.

Dr. Young (Closing). With respect to the point that Dr. Soderstrom brought up, there's no question that I agree with him a hundred percent that many things affect the outcome. It's been proved repeatedly, by multiple authors, that such factors as the degree of density of adhesions, the number of adhesions, and the integrity of the epitheal lining, besides the hydrosalpinx, all affect the outcome.

With respect to whether we have any laparoscopic lysis patients, we have a few who were not included in this study, but they are very few. Basically I have not been comfortable over the years with any heroic laparoscopic lysis of adhesions. If there are a few little adhesions, I think it's a great operation, but as the procedure begins to get trickier and I wonder where the ureter is and all that, I become "chicken." Thus most of our laparoscopic lysis cases have, in fact, been open cases.

Dr. Churches' comment is correct. All the major in vitro fertilization groups are reporting, except for England, where they keep pushing 30%, maximum pregnancy rates per laparoscopy of 15% to 20%, which I'm sure is no different statistically from these numbers that I'm showing you here. I suspect that at this point in time, you can reasonably look at in vitro fertilization as a 15% to 20% proposition and can make your calculations based on that.

In regard to Dr. Henderson's comments, the cases discussed herein represent only those cases in which disease was believed, after workup, to have resulted from infection. Our total microsurgery caseload for the study period, including sterilization reversals and other problems, was in excess of 200 cases, which I believe is more than enough to "keep our hands in." In any case, however, we do a modest number of these procedures, and the results as we show them are the results that we get.

The important point of this article, from my point of view, was to emphasize the applicability of both in vitro fertilization and microsurgery to the clinical private practice of infertility and to begin the process that we must all now go through, of deciding where the new in vitro fertilization technology fits into our more conventional treatment patterns. In vitro fertilization is no longer a "last ditch" or experimental tool. In answer to the last question, as to whether in vitro fertilization is applicable in less developed countries, the procedure is highly sophisticated and requires laboratory and physician support that, at least in the immediate future, I can only imagine being possible in highly sophisticated countries. I don't see it as something that's going to be imported to New Guinea, for example, in the near future.

Fetomaternal hemorrhage associated with umbilical vein thrombosis

Case report

Roger W. Hoag, M.D.
Berkeley and San Francisco, California

Antenatal thrombosis of the umbilical vein is usually a fatal condition for the fetus or neonate. The probable pathophysiology of this condition and its relationship to fetomaternal hemorrhage is discussed. A systematic study of the entire umbilical cord should be done when fetomaternal bleeding is diagnosed. (Am J Obstet Gynecol 1986;154:1271-4.)

Key words: Fetomaternal hemorrhage, umbilical vein thrombosis

Massive fetomaternal hemorrhage as the cause of perinatal mortality and morbidity occurs in approximately 1:800 deliveries and accounts for 3% of perinatal mortality. Massive fetomaternal hemorrhage has been defined as bleeding in which greater than 150 ml of fetal blood is found in the maternal circulation. Fetomaternal hemorrhage was found to be a cause of fetal death occurring before labor in 4 of 29 cases in which no other cause could be assigned.[1] Therefore a Betke-Kleihauer test should be done when an unexplained fetal death in utero or nonhemolytic anemia is found in the neonate. Thrombosis of the umbilical vein has been a condition only rarely recognized, with only 20 cases reported in the literature up to 1967. More cases have been added in the recent literature[2-5]; however, no report was found in which umbilical vein thrombosis and fetomaternal hemorrhage have been associated.

Case report

On a Sunday afternoon a 32-year-old woman, gravida 3, para 2, at 38 weeks' gestation in a totally uneventful pregnancy, reported having decreased fetal movement since that morning. A nonstress test was planned for the following morning. Twelve hours later a call revealed that she was still anxious about the relative lack of fetal movement, and she was admitted for observation. She had had no contractions, abdominal pain, or vaginal bleeding. She reported rust-colored urine without urinary urgency, frequency, or dysuria. Her past history was unremarkable, and her past obstetric history was that of two term spontaneous vaginal deliveries of two normal infants.

On the patient's admission to the hospital at 0100 hours (Monday), the fetal heart was observed to be steady at 150 bpm. The patient was having no contractions or vaginal bleeding, and the cervix was closed. The uterus was nontender on palpation. Blood pressure, pulse, and temperature were within normal limits. Observation of a voided admission urine specimen was cloudy yellow with no albumin present. A nonstress test was carried out; no accelerations were seen with two fetal movements observed by the nurse and the patient, and test results were reported as nonreactive. Nipple stimulation (contraction stress test) promptly induced uterine contractions, and the fetal heart exhibited many variable and some late decelerations. The nipple stimulation was halted, and the contractions gradually slowed to 6-minute intervals but lasted 70 to 80 seconds. Preparations were initiated for delivery by cesarean section. However, fetal bradycardia (90 bpm) developed and lasted several minutes, followed by a slow return to a baseline rate of 100 to 110 bpm but with loss of beat-to-beat variability. Review of the monitor records revealed the presence of a transient sinusoidal pattern in the nonstress test strip. Additional preparations were made for an emergency cesarean section; general anesthesia was induced, and 7 minutes later a pale, limp, 3.17 kg female infant with Apgar scores of 0 and 0 at 1 and 5 minutes was delivered. The amniotic fluid was meconium stained. There was no visible evidence of premature separation of the placenta, cord entanglements, forelying cord, compression, or hematomas. The umbilical cord appeared thin or empty. A cord blood sample was obtained with difficulty. Resuscitative efforts included intracardiac injection of epinephrine, which stimulated an irregular heart rate of 20 to 40 bpm to no avail, and the baby was pronounced dead

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after 30 minutes. The central hematocrit was 16%. Blood from the umbilical vein catheter revealed severe acidosis with a pH value of 6.46, P_{CO_2} of 143.3, P_{O_2} of 14.5, and base excess of −32. The mother's admission hemoglobin was 12.7 gm/dl, and a Betke-Kleihauer test of blood drawn before delivery revealed a 5.1% fetal hemoglobin, with an estimated fetal blood volume in the maternal circulation of 185 ml. Normal fetal blood volume calculated at 75% of body weight would equal 240 ml. A catheterized urine specimen (maternal) was negative for protein and contained many red blood cells, and 10 to 20 white blood cells per high, dry field. Urine culture yielded no growth. Maternal plasma hemoglobin was 0.7%, with a normal range in our laboratory of 0 to 5 mg/dl. Direct Coombs' test showed negative results. The mother's blood was type O, Rh positive, as was the baby's.

The placenta was meconium stained and exhibited a 30 ml adherent clot near a margin. Deep to the clot were several large infarcts, one of which was 2.5 cm in diameter; most were old, but one was recent. The cord was sectioned every 2 cm. In one section of the cord the umbilical vein was found to contain a thrombus adherent to the wall of the vein and completely occluded the vein. Microscopically there were leukocytes, platelet aggregates, and fibrin lines of Zahn in the thrombus, which was thought to establish a premortem onset of the thrombotic process of greater than 24 hours. Postmortem examination revealed an infant with a waxy pallor consistent with exsanguination with collapsed vessels; otherwise, gross and microscopic findings were within normal limits. No thrombosis of the ductus venosis was found.

Comment

When the umbilical vein is totally occluded, blood from the fetal circulation of the placenta has no possibility of return to the fetus. As blood from the fetal arterial side continues to be pumped into the placenta, the fetomaternal pressure differential increases across the hemochoriol membrane. It is postulated that this factor may contribute to fetomaternal transfusion by producing leakage or may increase the pressure for leaks in areas of preexisting lesions or damage to endothelium and syncytial membrane at their junction with decidual tissue. However, umbilical vein thrombosis associated with fetal anemia and a failing circulation could be due to stasis of flow, which is a known factor in producing or adding to a thrombus. Otherwise, in the absence of compression, torsion, trauma, or hematoma, the thrombosis is unexplained. Even if the thrombosis resulted from stasis of circulation, it may still have contributed to the downward spiral of anemia, hypovolemia, distress, acidosis, and death.

Testing for fetomaternal hemorrhage should be done in every case of unexplained fetal death or nonhemolytic anemia in the newborn. It is additionally recommended that careful longitudinal opening of the umbilical vein or sectioning of the cord every 1 to 2 cm should also be done to search for thrombosis of the umbilical vein. This condition may, in fact, be present more often than was previously recognized.

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Discussion

DR. JOHN J. MOLITOR, Fullerton California. Dr. Hoag has presented us with two fascinating mishaps of fetal circulation. One, umbilical vein thrombosis, is exceedingly rare, as he has pointed out. The other, fetomaternal hemorrhage, is more common than most of us realize and is even more commonly unrecognized. Many obstetricians are not familiar with massive fetomaternal hemorrhage. Usually the two events have been reported as occurring separately. In Dr. Hoag's case they were associated in the same patient. The extreme rarity of this association precludes a meaningful discussion based on statistics or reports of past experience because there simply are none. Dr. Hoag postulates that occlusion of the umbilical vein increases the pressure in the fetal vascular spaces of the placenta, predisposing or causing leakage of fetal blood into the maternal vascular compartment. This hypothesis certainly is attractive and seems plausible just on the basis of hydraulics. If it is operative, however, it is uncommon and leaves unexplained the cause of the much more common and often lethal massive fetomaternal hemorrhage. Today anything that causes fetal distress or intrauterine fetal death will command the attention of the obstetrician as never before. In addition to our scientific curiosity, another reason compels our attention. The reason, of course, is the difficult and distressing medicolegal climate in which we work. I first became interested in this problem when I had a case, approximately 5 years ago, that resulted in a brain-damaged baby and, of course, a lawsuit. Such an experience is guaranteed to stimulate your interest.

The Europeans have recognized and written about massive fetomaternal hemorrhage for years, but comparatively little has been written in the American literature. The reason is unclear, but I suspect it is that the focus has been on other, more common problems that also have a potentially greater economic impact, such as diabetes complicating pregnancy, uteroplacental insufficiency, intrauterine growth retardation, and pregnancy-induced hypertension. The last three of these problems might conceivably have a cause in com-

mon with each other and possibly with fetomaternal hemorrhage as well—as recent studies of placentation have indicated.

Paradoxically, the bleeding of the fetus into the mother holds the greatest clinical significance for the obstetrician at the two extremes of the magnitude of the bleeding. It has been known for years that fetal blood cells get into the maternal circulation. Weiner[1] first proposed, in 1948, that transplacental hemorrhage of fetal Rh-positive red blood cells into the circulation of the Rh-negative mother was the cause of Rh isoimmunization. This proposal was confirmed in 1954 by Chown,[2] who reported the delivery of a very anemic Rh-positive baby from an Rh-negative primipara. The mother was found to have 5% Rh-positive fetal red blood cells in her circulation, and she promptly developed an Rh antibody response. It has been shown subsequently that as little as 0.1 ml of fetal blood can cause an antibody response in the mother. Fetomaternal bleeding in the range of 30 to 40 ml can cause antibody response but usually does not seriously impair fetal hemodynamics. The massive fetomaternal hemorrhage, over 150 ml, can kill the fetus or seriously compromise its circulation.

The acid elution test developed by Kleihauer and associates in 1957 and now known as the Betke-Kleihauer test is a very accurate, sensitive method of differentiating fetal red cells from adult red cells and allows the quantitative evaluation of fetal erythrocytes in the maternal circulation. It has been of great value in determining the incidence and size of fetal transplacental hemorrhage at various gestational ages, at delivery, and following various obstetric procedures and complications.

Approximately 50% of gravidas have evidence of fetomaternal bleeding during pregnancy or immediately after delivery. The significance of these small fetomaternal bleeding episodes, as we all know, lies in their immunologic potential. Failure to recognize the possibility of these small amounts of bleeding and to initiate measures to prevent sensitization may leave the physician open to legal action in the event that the mother is sensitized, because once the damage is done, it cannot be undone. The cause of massive fetomaternal hemorrhage in most of the reported cases has been unexplained. Reported specific causes include placenta previa, abruptio placentae, trauma, and manual removal of the placenta. In recent years, amniocentesis has become one of the most common causes, and external version has also been reported as a cause.

In the literature I reviewed, most of the babies in these cases died antepartum or very soon postpartum, as was true, in Dr. Hoag's case. I did not find any reports of infants' surviving with significant brain damage. The majority of these cases were apparently acute massive fetomaternal hemorrhage, not chronic. I wish to ask Dr. Hoag whether he has noted the same thing and whether he has found any cases of cerebral palsy, seizure disorders, or impairment of intellectual function attributed to fetomaternal hemorrhage. The more evidence we accumulate to show that damage to the baby's central nervous system can occur before labor, the less vulnerable we will be to lawsuits and their astronomic awards.

Out of curiosity I asked the medical records department in my hospital to pull all cases of fetomaternal hemorrhage for me. Data from only the last 3 years have been entered into the computer. There were no cases of fetomaternal hemorrhage. I next asked for the records of all cases of nonhemolytic anemia in the newborn. Again, there were no cases. I then asked our blood bank how many Betke-Kleihauer tests had been performed in the last 3 years and whether any of them had revealed massive fetomaternal hemorrhage. Fifteen tests had been performed in the previous 40 months, during which time there were 7696 live births. Seven tests showed significant bleeding, the largest being approximately 110 ml. There was no bleeding over 150 ml. Four of the tests were ordered by one obstetrician following elective external cephalic version. I think it is apparent that in my hospital, at least, massive fetomaternal hemorrhage is a very infrequent occurrence or is being missed. I don't know how many unexplained fetal deaths there were in that period, but obviously nobody considered massive fetomaternal hemorrhage as a possible cause, since no one looked for it.

I agree with Dr. Hoag's recommendation that in each case of massive fetomaternal hemorrhage, the umbilical cord should be carefully sectioned to look for thrombosis of the vein. His presentation's greatest value in my mind, however, is that it again directs our attention to the fact that the fetus can bleed into the mother in large amounts and without any external signs that anything is amiss. It is an occurrence that will not be diagnosed if it is not thought of and looked for. Although it may not be possible to materially improve the salvage rates, we should be able to explain some previously unexplained fetal deaths and to absolve the obstetrician of any responsibility for them.

A by-product of my preparation for this discussion was that I delved into the literature on placental pathology, looking for a lesion that would account for this bleeding. In a majority of the cases, as previously mentioned, no placental lesion was found. Reading some of the more recent literature, I found that there is a renewed interest in placental study, with attention directed to the uterine side of the placenta to look for possible defects that might explain otherwise the inexplicable pregnancy complications. Preeclampsia and intrauterine fetal growth retardation are two such disorders in which new information has come to light by the study of placental bed biopsies and occasional cesarean hysterectomy specimens. Older concepts are being discarded, especially concentration on functionally unimportant gross abnormalities, and new emphasis is being placed on more subtle pathophysiologic processes. Such mysteries as preeclampsia and intrauterine growth retardation may actually be due to disorders of placentation in its earliest stages. I quote H. Fox[3] in a recent publication:

The placenta is a vigorous, energetic, versatile organ with a considerable physiologic reserve capacity. Most pathologic lesions of the placenta are functionally irrelevant and it is doubtful if the placenta often, or ever, becomes insufficient. Attention is being increasingly directed to the interaction of extravillous trophoblast and maternal tissues during placentation and it is becoming clear that many of the ills that beset a pregnancy have their origin in an abnormal interrelationship between fetal and maternal tissues during the earliest stages of establishment of gestation.

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DR. JULIAN PARER, San Francisco, California. I've got two comments. The first is that I know of at least one case of maternofetal bleeding, with a subsequent newborn hematocrit value of 15%, in which there was no sinusoidal pattern at all. And I think it is of some interest that your case also showed a flatness of baseline, as well as a classic sinusoidal pattern. I don't know where this fits into the cause of this sinusoidal pattern, but I just leave you with the comment that it's not always a constant thing.

Now, the second comment I'd like to make is with respect to the tolerance of the fetus for anemia. In slowly developing anemia, as in erythroblastosis, we've noticed fetal hematocrit values, or newborn hematocrit values subsequently projected back to fetal, down to 8%. This is still consistent with intrauterine life and consistent with intrauterine pneumonia, as demonstrated by cord blood gas values. The fetus has a remarkable resistance to slowly developing anemia. These fetuses can still move—not too many of them at such a low hematocrit value, but certainly with a hematocrit of 15% the fetuses can still move. In fact, they may have a respectable biophysical profile, which I think is an indication that the use of this stress test is much more important in these babies who are at risk for severe anemia.

DR. RICHARD PAUL, Los Angeles, California. The case discussion seems to indicate that the patient had noticed symptoms that brought her initially to the hospital some hours before. Then if one speculates about the occlusion of the vein and the possible consequence of that—and then the subsequent patterns and the abrupt problems at the end—the cause was probably hypovolemia. I wonder whether there was any finding at autopsy in the baby's heart. It might relate either grossly to failure or microscopically to some lesions that may have developed during this process, in addition to the findings in the umbilical vessels.

DR. ROBERT GOODLIN, Denver, Colorado. What was the time interval between what looked like a "normal baseline," although sinusoidal, and the delivery of the fetus? I had an unfortunate case in which an amniocentesis showed bloody fluid and in which the fetal heart rate looked worrisome. I thought that we had a relatively adequate amount of time, but I, likewise, delivered a stillborn infant. I had always thought that there would be a time of profound bradycardia before the occurrence of fetal death.

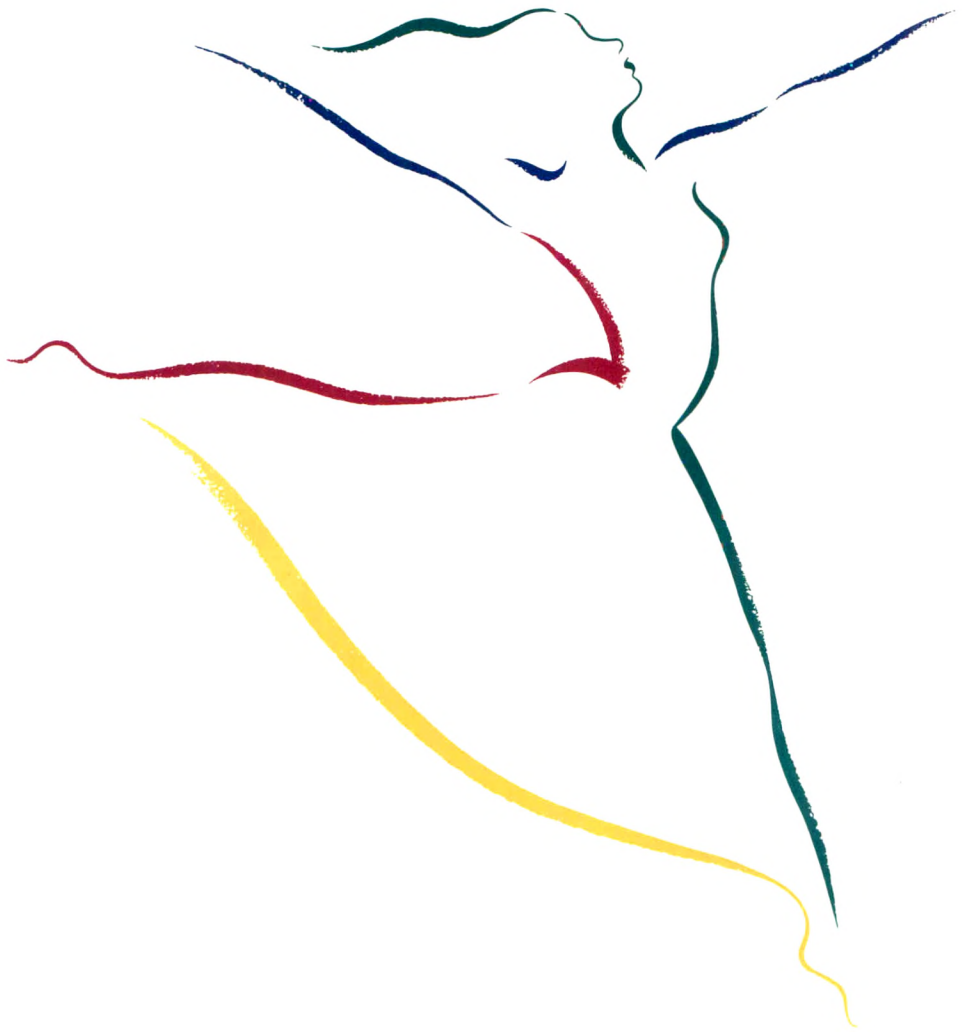
I also wish to comment that, at least in the literature now, there are a fair number of reports of nonimmune hydrops. This problem can also result from fetomaternal hemorrhage and is something we should look for.

DR. HOAG (Closing). One question asked was whether I had observed, in my search of the literature, any correlation of mental retardation, cerebral palsy, etc., with massive fetomaternal bleeding. I did not address that question, and therefore I have no information on that score, although it seems to me, just speculating, that if the fetus were in severe distress over a period prior to delivery, the infant would not be immune to such an outcome.

Dr. Paul, the pathologist's only comment about the child's heart was that the chambers were small, a finding consistent with hypovolemia. He did not find myocardial infarction. I don't know how carefully he looked for it, but he didn't diagnose it.

In reference to Dr. Goodwin's question on the time lapse—this patient was admitted at 1 o'clock in the morning, and we began the process of monitoring her. We delivered her at roughly 5:40 AM, approximately 4 hours and 40 minutes later. The course of events escalated rapidly, and in the absence of any prior history, symptoms, or signs, one found the situation hard to believe, especially at 2 or 3 o'clock in the morning. As the situation deepened and worsened, the tempo increased and led to the emergency cesarean section, which was probably performed too late. Whether the results would have been different—or maybe even worse—if we had operated an hour sooner, I don't know.

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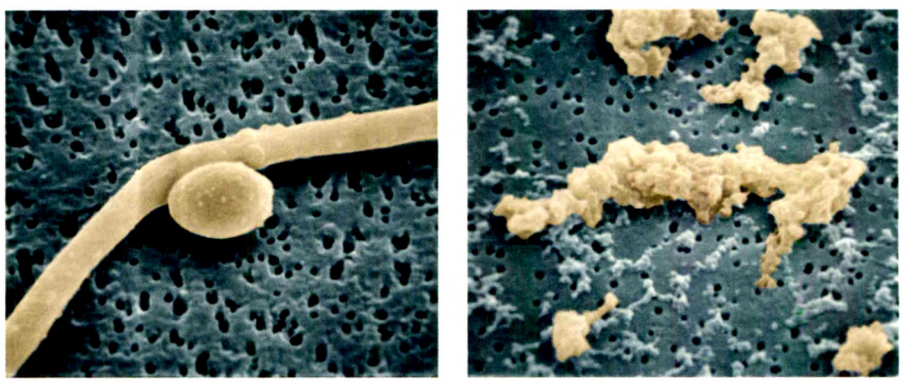
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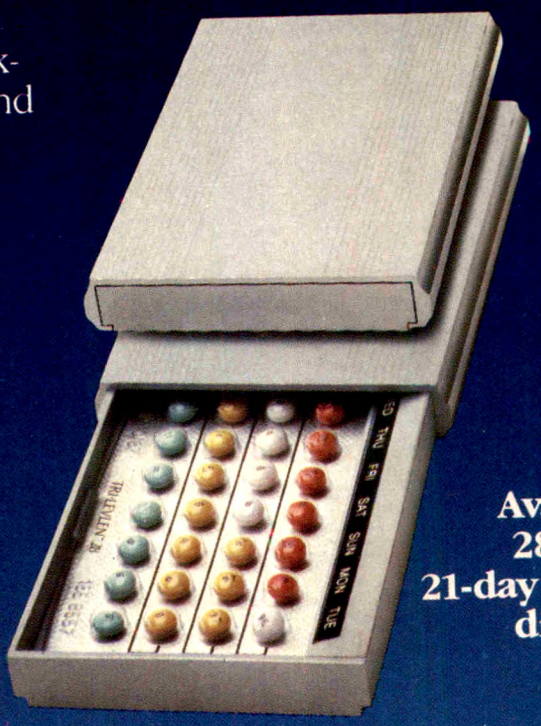
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Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen™—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000); the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule: OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information and package insert.

References: 1. Data submitted to FDA. **2.** Skouby SO, Kuhl C, Mølsted-Pedersen L, et al: *Am J Obstet Gynecol* 1985;153:495-500. **3.** Vermeulen A, Thiery M: *Contraception* 1982;26:505-513.

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430-27

Preinduction cervical ripening with sequential use of prostaglandin E_2 gel

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Two hundred patients received a prostaglandin E_2–containing gel for preinduction cervical ripening. The gel was administered every 6 hours until a Bishop score ≥7 was obtained or the attending physician deemed induction necessary. Forty-eight percent of the patients labored "spontaneously" after one or more gel applications. The average cumulative change in Bishop score with sequential application of the gel was 2.7. Sequential application of the prostaglandin gel proved no more effective in the process of preinduction cervical ripening than single application of the gel with a similar period of observation. (Am J Obstet Gynecol 1986;154:1275-9.)

Key words: Prostaglandin gel, cervical ripening

Prostaglandin-containing gels for the purpose of cervical ripening are now in widespread use.[1-5] Optimum methods of application, however, have yet to be determined. Previous studies by our group[6,7] using prostaglandin-containing gels determined that cervices ripened to a Bishop score of ≥7 had a much higher incidence of induction success and a lower occurrence of cesarean section. The protocol followed in these investigations allowed for one gel application followed by a ripening period of 12 to 16 hours and subsequent Pitocin induction of labor regardless of cervical Bishop score. On the basis of these studies it was felt that a sequential application of prostaglandin-containing gel might further ripen the cervix and enhance the effectiveness of prostaglandin gel use.

Methods and material

Prostaglandin gel was prepared as previously described.[6] The final concentration was 5 mg of prostaglandin E_2 per 10 ml of gel.

The study group consisted of 200 patients carrying singleton pregnancies and needing induction of labor for medical indications. The patients were assessed before gel instillation on the morning of the day of induction. To reduce bias in the estimation of the Bishop score two examiners evaluated each patient before and 6 hours after each gel application. The average of the two scores constituted the assigned Bishop score.

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After the examination 10 ml of gel (5 mg of prostaglandin E_2) was instilled into the vaginal fornices with a No. 20 red-rubber catheter. The catheter was then withdrawn and the patient remained recumbent for 15 minutes. Maternal vital signs were assessed every 4 hours. Uterine contractions and fetal heart rate were monitored continuously with an external fetal monitor.

The patient was reevaluated every 6 hours after the initial gel application. If the averaged Bishop score was ≤6, gel was reapplied. This protocol was repeated until a Bishop score of ≥6 was achieved or the attending physician determined induction necessary. Oxytocin administered by intravenous infusion pump was then begun. Adequate oxytocin stimulation was defined as firm, regular, painful contractions with an average frequency of one every 3 minutes. Membranes were ruptured with the onset of active labor, defined as progressive cervical dilatation. Failed inductions were defined as those cases where adequate labor could not be established or those where, although adequately stimulated, no progressive cervical change ensued. All data were analyzed statistically by Student's t test or χ^2 with the use of the Edu-Ware Statistics Package 3.0.

Results

The 200 patients consisted of 109 primigravid and 91 multigravid women with an average gestational age of 39.9 weeks. The average gel-induction interval for the study was 14.9 hours; the average Pitocin dose, 13.2 mU/min; and the average length of labor, 8.0 hours. The rate of maximum dilatation in the active phase (used as a clinical assessment of cervical compliance) averaged 4.7 cm/hr. "Spontaneous" labor rates for sequential gel application are shown in Table I. Gel application alone accounted for an induction rate of almost 50%. The average pregel Bishop scores and in-

Table I. Spontaneous labor

First dose		*Second dose*		*Third dose*		*Total*	
n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
83/200	41.5	12/41	29	1/17	5.9	96/200	48

Table II. Cervical change

Pregel score	*Change Number 1*	*Change Number 2*	*Change Number 3*	*Total change*
3.9	1.9	1.3	1.0	2.7

Table III. Influence of gestational age

Gestational age (wk)	*Pregel score*	*Total change*	*Spontaneous labor*		*Pitocin dose*	*Maximum dilatation rate (cm/hr)*	*Cesarean section*	
			n	*%*			*n*	*%*
<37	3.4	3.2	4/18	22	11.2	6.4	2/18	11.1
37-42	4.0	2.7	91/156	51.9	14.2	4.8	32/156	20.5
>42	4.0	1.9	11/26	42.3	9.5	3.2	9/26	34.6

Table IV. Spontaneous versus induced labor

Labor	*Pregel score*	*Length of labor (hr)*	*Maximum dilatation rate (cm/hr)*	*Cesarean section*	
				n	*%*
Spontaneous (n = 96)	4.2	7.5	4.8	14	14.6
Induced (n = 104)	3.7	8.7	4.7	29	27.9
T	2.23	1.81	NS	5.23	
p Value	<0.025	<0.05		<0.025	

Table V. Influence of parity

Parity	*Pregel score*	*Total change*	*Spontaneous labor*		*Maximum dilatation rate (cm/hr)*	*Cesarean section*		*Pitocin dose*
			n	*%*		*n*	*%*	
Primigravid (n = 109)	2.9	2.8	43/109	39	3.7	27/109	24.8	13.6
Multiparous (n = 91)	4.0	2.5	53/91	58	5.9	16/91	17.6	12.6
p Value	NS	NS	<0.01		<0.005	NS		NS

Table VI. Cephalopelvic disproportion versus normal labor

Labor	*Pregel score*	*Total change*	*Spontaneous labor*		*Maximum dilatation rate (cm/hr)*	*Infant weight (pounds)*	*Pitocin dose*	*Primigravid*		*Multiparous*	
			n	*%*				*n*	*%*	*n*	*%*
Cephalopelvic disproportion (n = 29)	4.0	2.6	9/29	31	1.2	8.7	13.2	20/29	69	9/29	31
Normal (n = 161)	3.9	2.7	87/161	54	5.1	7.5	13.2	83/161	52	78/161	48
p Value	NS	NS	<0.05		<0.005	<0.005	NS	NS		NS	

Table VII. Failed inductions

Induction of labor	Primigravid		Multiparous		Gestational age (wk)	Pregel score	Total change	Pitocin dose
	n	%	n	%				
Failure	6	60	4	40	39.4	3.4	1.3	23.7
Success	103	54	87	46	39.8	4.0	2.8	12.5
p Value	NS		NS		NS	<0.05	<0.025	<0.025

terval Bishop scores are shown in Table II. The change in Bishop score was assessed only in those patients not laboring "spontaneously." There were 43 cesarean sections performed in the study group for a cesarean section rate of 21.5%. Twenty-nine of these (14.5%) had as the indication cephalopelvic disproportion; 12 (6%), fetal distress; and two (1%), other indications.

Cervical ripening and labor outcomes differed with gestational age. While there was greater cervical change in the group <37 weeks' gestation, there was a much lower incidence of "spontaneous" labor. The least cervical change occurred in the group >42 weeks' gestation. This group also had the lowest maximum rate of cervical dilatation in the active phase and the highest rate of cesarean section. The proportions of primigravid and multigravid women did not differ significantly among the groups (Table III).

Those patients laboring "spontaneously" after gel application had, on the average, higher initial Bishop scores, shorter labors, and a much lower rate of cesarean section (Table IV).

Primigravid women differed from multigravid ones only in having a lower incidence of "spontaneous" labor after gel application and a lower maximum rate of dilatation in the active phase (Table V).

Those patients undergoing cesarean section for the indication of cephalopelvic disproportion, when compared with all normally laboring patients, had a lower incidence of spontaneous labor after gel insertion, a much lower maximum rate of cervical dilatation in the active phase, and higher infant birth weights (Table VI).

Those patients classified as having failed inductions differed from the remaining study group by having lower pregel Bishop scores, less cervical change, and a much higher dose of oxytocin required to produce adequate labor (Table VII).

There were no maternal or fetal-neonatal deaths in the study group. Maternal morbidity consisted of 16 cases (8%), detailed in Table VIII. Infection and hemorrhage accounted for most of the morbidity. There were five instances of neonatal morbidity: three cases of Erb's palsy, one case of meconium aspiration, and one case of respiratory distress and bronchopulmonary dysplasia in a 1 pound, 8 ounce infant.

Table VIII. Maternal morbidity
(n = 16/200, 8%)

Complication	n
Postpartum hemorrhage	7
Infection	4
Uterine window	1
Abruptio placentae	1
Retained placenta	2
Thrombophlebitis	1

When the outcome of patients in the current protocol was compared to that in our previous study with the use of only one gel application and a 12- to 16-hour delay before induction,[6] no significant differences could be found. Total cervical change remained similar (2.5 versus 2.7) as did the incidence of "spontaneous" labor after gel insertion (46% versus 48%). Failed inductions accounted for 6% of the cases with single gel use and 5% of the cases of sequential gel use. The incidence of cesarean section did not differ significantly.

Comment

The aim of the present study was to assess the efficacy of sequential vaginal prostaglandin gel application in ripening the cervix and promoting successful labor induction. Our results confirm the gel's effectiveness when used in this manner. However, when the results were compared with those of our earlier study, sequential gel application appeared no more effective than single gel application followed by an equal period of observation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Hedric B. Hanson, Anchorage, Alaska. The problem of medical induction of labor when there is an unfavorable cervix continues to perplex the clinician. The data in the world literature seem to support the premise that the application of prostaglandin E_2 to the cervix promotes cervical ripening and facilitates the induction of labor. Shepherd et al.,[1] in 1981, presented 1000 consecutive unselected cases of induction of labor with the use of prostaglandin E_2 suppositories for medical or obstetric reasons. Only 27% of the entire series required augmentation of labor with intravenous oxytocin, the range being from 9.9% of multigravid women with a favorable cervix to 42.4% of primigravid women with an unfavorable cervix. With an unfavorable cervix, 42% of the primigravid women versus 25% of the multiparous patients required a second inducing suppository. Prostaglandin E_2 suppositories have become routine at London's Queen Charlotte Maternity Hospital, being simple, safe, and highly acceptable to the patients and obstetricans alike in all cases in which a simple amniotomy would not suffice. The overall rate of failed inductions resulting in cesarean section in those patients with an unfavorable cervix has fallen from 42% to 2.5%.[1] Ekman's group[2] in Malmo, Sweden, has demonstrated, in a randomized study, that patients with a highly unfavorable cervix apparently respond best to intracervical application of the prostaglandin E_2 gel. With a relatively ripe cervix there are small differences in efficacy between the intracervical and intravaginal routes of administration. However, this same group warns of potential maternal and fetal side effects that are particularly pronounced with the intravaginal administration of prostaglandin E_2. That intravaginal administration of prostaglandin E_2 gel induces considerable myometrial activity compared with that produced by intracervical administration was objectively confirmed by the intrauterine pressure recordings. The Ekman group achieved cervical ripening, induction of labor, and delivery with an 8% cesarean section rate.[2]

The prostaglandin E_2 gel has not been approved by the Food and Drug Administration. Experimental protocols have been developed at various university and community hospitals. Macer et al.[3] have presented data on the use of 3 mg prostaglandin E_2 suppositories obtained by cutting a 20 mg prostaglandin E_2 suppository into 3 mm chips. The Portland group has documented the effects of prostaglandin E_2 vaginal gel prepared by thawing the 20 mg prostaglandin E_2 vaginal suppository and homogeneously mixing it with a methylhydroxyethyl cellulose gel (tylose). This preparation ripens the cervix, reduces induction failures, diminishes oxytocin dosage needed for induction, and lowers the cesarean section rates.[4] The present article is a companion study of and demonstrates that the sequential application of the prostaglandin E_2 gel appears no more effective than single gel application.

I, however, have some reservations about the study design. The study was designed to evaluate the effects of sequential application of prostaglandin E_2 on cervical ripening. Considering the sequential application of prostaglandin E_2, Why was the intravaginal route chosen over the intracervical route? Why were the second and third applications not placebo-controlled? Considering the cervical ripening, how was the interobserver reliability (Bishop score) studied? Are there more objective methods of evaluating cervical ripening? Was the modified Bishop score proposed by Lange considered? Was sonographic evaluation of the lower uterine segment and cervical changes considered?

Despite my questions, I would again like to emphasize the importance of the work done by the Portland group in documenting the effects of the reconstituted prostaglandin E_2 gel on cervical ripening.

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Dr. Robert Sack, Whittier, California. Dr. Prins, I am concerned about one thing. There were 16 complications, and three of them were cases of Erb's palsy, that is, 70% of the complications were Erb's palsy. I would like your response.

Dr. William Graves, San Francisco, California. Dr. Prins said these were all medically indicated inductions, and I think the value of the written article, at least, would be enhanced if he could break these down a little bit for us. I would suggest he use the same two observers when the gel is applied sequentially. If we are going to present any studies on the induction of labor, probably we really should separate parous patients from primiparous patients. The real value of anything that can enhance labor is particularly going to be present in the patients at 42 weeks' gestation, and I hope that the written article will establish this group and

evaluate it separately. This is a worthwhile study on a very important obstetric subject.

Dr. E. Paul Kirk, Portland, Oregon. Dr. Prins emphasizes the well-known fact that difficult things in obstetrics are difficult to deal with.

I was interested that even in those two subgroups where it might appear that the outcome would be more advantageous, namely, those patients who went into "spontaneous labor" after prostaglandin administration and the multiparous group, there were high cesarean section rates, one of 14% and one of 17%. These findings are somewhat inconsistent with other reports for those subgroups, and I wondered whether there was any explanation for that. I would like to ask specifically whether women who had a previous cesarean section were excluded from this form of induction.

Dr. Edward Quilligan, Sacramento, California. Hypertonus has been frequently mentioned as a complication of vaginal prostaglandin. Would Dr. Prins discuss the frequency of hypertonus and whether any instances of fetal distress were associated with hypertonus.

Dr. Prins (Closing). The study design of intravaginal gel was designed essentially to compare sequential versus single use. In previous studies we looked at whether the gel was effective and performed studies in which we used placebo gels matched against prostaglandin E_2–containing gels. We established that, in our hands and with our preparation, the gel appeared to work. That is the reason we did not pursue any placebo-controlled experiments.

Ulmsten et al. have shown that intracervical application of 500 μg of prostaglandin E_2 is probably more effective than vaginal application. It is, however, more difficult to do. We currently have about 100 patients in our study of intracervical application versus intravaginal application.

The reason for using the older Bishop score was twofold. First, it has allowed me to compare my data with those of the European investigators, who until the early 1980s were the only people working with prostaglandin gel as a ripening tool. Second, it allowed me, once I started, to compare my data, as I have done here, from one study to the next. That is the rationale for the continuation of the perhaps outmoded Bishop scoring system.

The issue of ultrasound is an interesting one. It certainly has been used for diagnosis of incompetent cervices, but the cervix, with as much water content as it has at term, does not lend itself well to this modality. Although I think dilatation and effacement could be seen to a degree, the measurements, although more precise, would be no more accurate in determining whether the cervix is ripe or not ripe.

We had three cases of Erb's palsy in 200 patients. It was indeed three of the 16 in our complication group and I think reflects the fact that we were inducing a large number of high birth weight postterm infants.

The majority of our patients had labor induced because of postdatism; large for gestational age infants; diabetes, insulin-dependent or non-insulin dependent; hypertension, pregnancy-induced or chronic, and other maternal indications.

I had hoped that the examiners would be the same, but they were not. I do not have absolutely accurate records, but at least 60% of the time the same two people examined the patient, and there was always one person in common for each examination. When the 42-week group was separated out, we found that they had a high rate of cesarean section, less cervical change, and a much lower rate of cervical change in the active phase. The "high rate of cesarean section" in the study is something that we simply have to live with. In our initial study of prostaglandin in a population that was high risk with unfavorable cervices and with the use of placebo controls, our cesarean section rate was around 40% in the Portland area. I do not know whether that is right or wrong but it is a fact. With the use of prostaglandin gel in these difficult circumstances, our cesarean section rate has almost been halved. Whether it can be changed more than that I do not know.

Dr. Kirk picked up on the fact that there was one repeat cesarean section in the group, which I had included in the complications. We have a separate protocol in Portland for using prostaglandin gel for preinduction cervical ripening in cases of prior cesarean section. I personally think it is an ideal tool for this. This woman was inadvertently entered into the study and I did not feel it was fair to remove her. She was gravida 2, para 1, with a prior cesarean section. She did not have spontaneous labor with the gel and underwent approximately 8 to 9 hours of Pitocin stimulation. A repeat cesarean section was performed because of cephalopelvic disproportion, and she was found to have a uterine window at the time of cesarean section.

There were no cases of hypertonus in these 200 patients. We have had cases of hypertonus in Portland when the gel was used, and they have occurred with what I would term the injudicious use of the gel in women with very ripe cervices (Bishop scores of ≥ 8) and in grand multiparous patients with very ripe cervices. Before the gel was well established for use in the Portland area, Pitocin and gel were on occasion used together. The combination of oxytocin and postaglandin gel is almost a guaranteed hypertonus situation.

On the origin and progression of human breast cancer

Hanne M. Jensen, M.D.
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The mature human female breast contains thousands of hormone-sensitive, potentially milk-producing microorgans, called lobules. In the nonpregnant state they vary in size from 1 to 8 mm but most are 1 to 2 mm in diameter. Each lobule is drained by a terminal duct attached to the main duct system. It is called the terminal ductal-lobular unit, which normally regresses at menopause. Most breast diseases except papillomas in major ducts arise in terminal ductal-lobular units. Disease processes such as hyperplasias and neoplasia alter the terminal ductal-lobular units to such a degree that conventional light microscopic appearances make the structures appear as though they were ducts. However, the basic microarchitecture in three dimensions is preserved, proving the origin of cancer is the terminal ductal-lobular unit. (Am J Obstet Gynecol 1986;154:1280-4.)

Key words: Breast cancer, origin, progression

Development of breast cancer is a common occurrence in the human female. It usually presents as a firm mass and most often is detected by the woman herself. At this time the disease has usually progressed to the invasive stage when cure is less likely. How can the obstetrician and gynecologist who cares for women in their younger years of life, when the disease is in a precancerous phase, possibly detect the women at risk? Mammography is valuable because minute irregular calcifications are seen in the x-ray films, attesting to focal areas of necrotic tissue that have imbibed calcium. Such calcifications occur within foci of ductal carcinoma in situ, the preinvasive stage of the disease.

Might it some day be possible to detect the disease in its precancerous stage and intercept it with some form of treatment so that the illness would be arrested at this phase of development? What is known about the origin and progression of breast cancer?

In rodents the mammary fat pads are so thin that they can be stained in toto and the fat can be dissolved so that an entire gland with its branching duct tree can be seen beautifully in three dimensions. It occurred to Dr. Sefton Robert Wellings who had studied precancer in the mouse mammary gland for many years that a study on the microarchitecture of the human mammary gland might elucidate new knowledge about breast cancer in women. Dr. Wellings obtained a contract from the Breast Cancer Task Force in 1967. I joined his research program in 1969 and remained with the study until 1975.

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Reprint requests: Dr. Hanne M. Jensen, Department of Pathology, School of Medicine, University of California, Davis, Davis, CA 95616.

Material and methods

Human mammary glands from 67 autopsies, in which no history of breast disease was noted, served as controls. Fifty-two mastectomy specimens with cancer or contralateral to a previous breast cancer constituted the study group.

Formalin-fixed, whole breasts were sliced on a butcher's meat slicer into 2 mm thick slices in the parasagittal plane. The slices were stained with hematoxylin, washed in water, dehydrated in increasing concentrations of ethyl alcohol and acetone, and finally impregnated with methylsalicylate to diminish light refractions. After the slices were placed in plastic bags containing methylsalicylate, they were viewed at two to 10 times magnification in a dissecting microscope.

Any abnormal structure that had a three-dimensional appearance different from the normal was isolated from the slice and photographed in its entirety at 2 to 10 times magnification. The abnormal structure within the isolated piece was studied under the light microscope after conventional processing for light microscopy with paraffin embedding, 7 μm sectioning, and staining with hematoxylin-eosin stains. The microarchitecture seen three-dimensionally in the photograph was compared and correlated with the histopathologic structure seen in two dimensions in the light microscope. We thus learned that the origin of breast cancer is distal to the branching duct tree.[1] Since all lesions within a given breast were studied, content of disease could be measured in quantity and quality.[2] A grading system for lesions deemed precancerous was devised.[2]

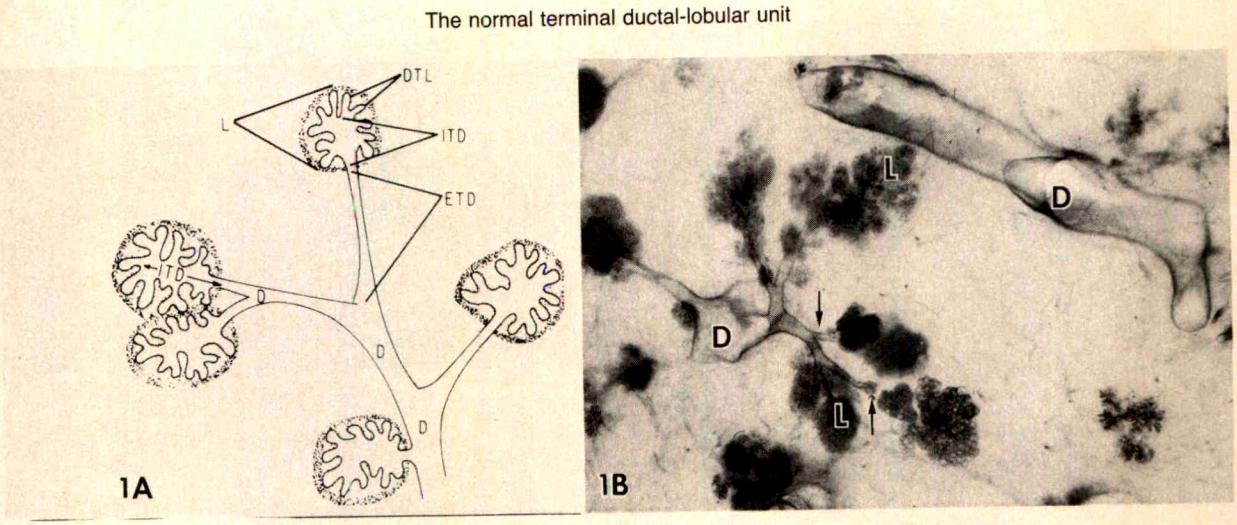


Fig. 1. *A*, Schematic drawing. Ductules *(DTL)* arise from the intralobular terminal duct *(ITD)*. The extralobular part of the terminal duct *(ETD)* connects to the duct tree *(D)*. *B*, Breast slice. *DTL + ITD* constitute a lobule *(L)*. *Arrows* point to *ETD* draining into *D* (×5).

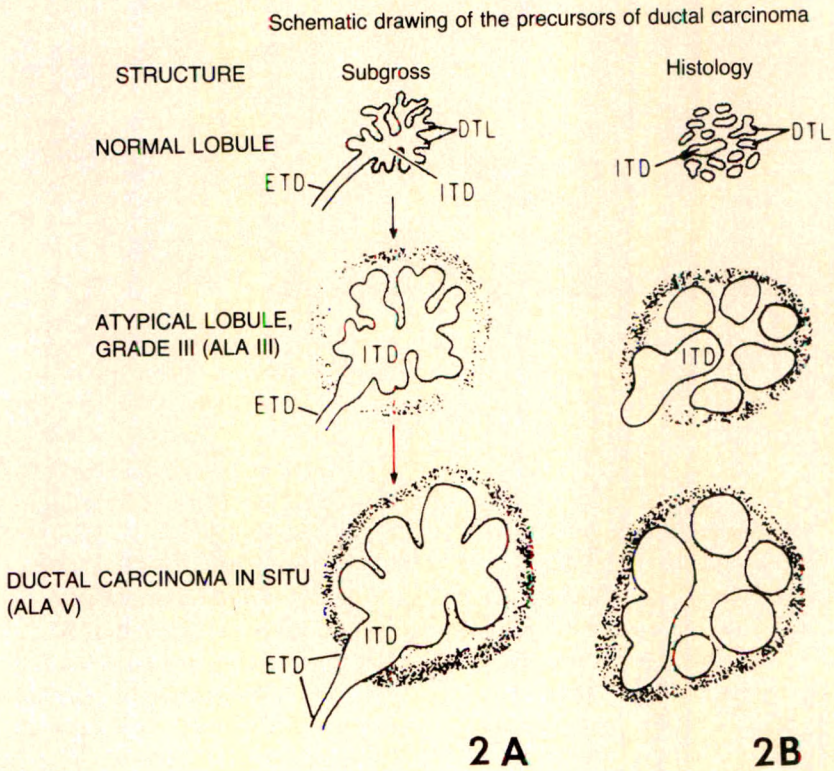


Fig. 2. *A*, The delicate ductules become increasingly coarser as a lobule becomes increasingly more atypical. The terminal ductal-lobular unit is still preserved as a distinct entity. *ALA*, Atypical lobules type A. *B*, In the corresponding histologic two-dimensional appearance the ductules eventually reach the size of small ducts. In such preparations it is hard to appreciate that each constellation in fact represents a terminal ductal-lobular unit.

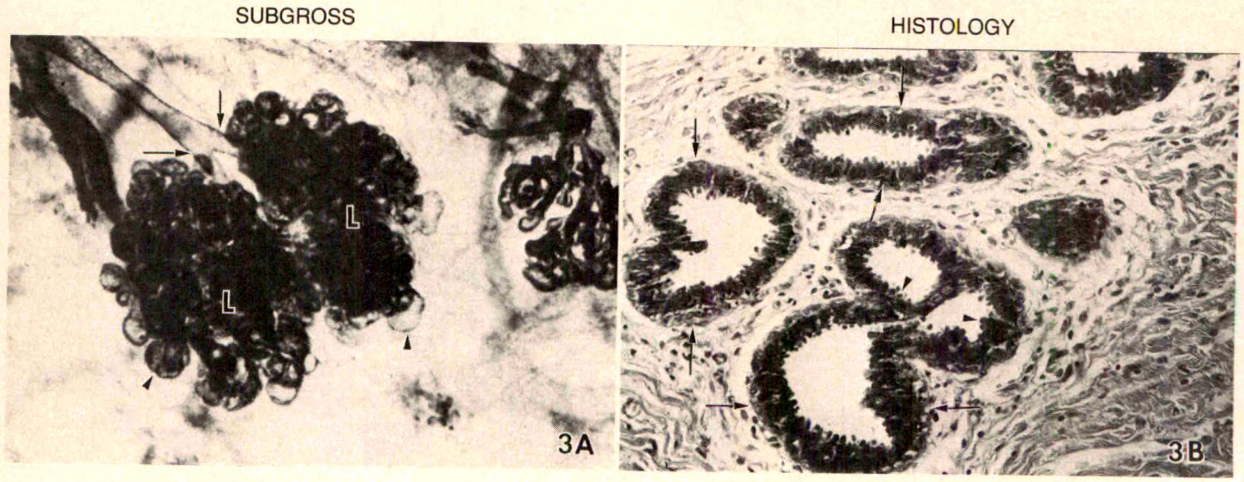


Fig. 3. *A*, ALA I. *Arrows* point to extralobular terminal ducts of two lobules *(L)*. *Arrowheads* point to individual ductules. (×13.) *B*, ALA I. Several ductules outlined by *arrows*. *Arrowheads* point to apical cytoplasmic blebs next to the lumen of each ductule. (×125.)

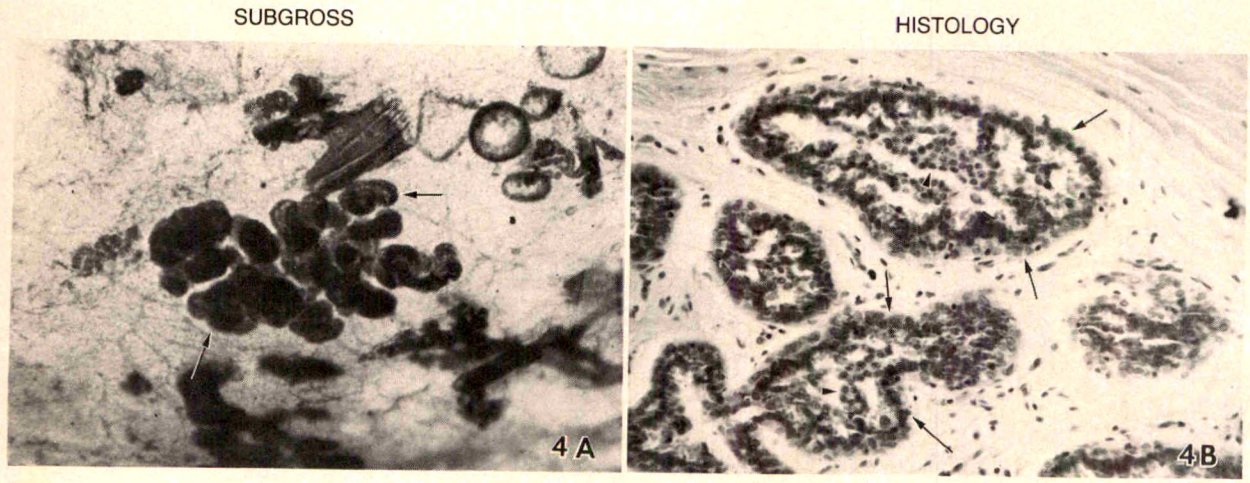


Fig. 4. *A*, ALA III. *Arrows* point to cellular ductules of the terminal ductal-lobular unit. (×16.) *B*, ALA III. Several ductules outlined by *arrows*. *Arrowheads* point to cells filling the lumen of each ductule. (×125.)

Results

The microarchitecture of the normal breast parenchyma is very beautiful. The lobules cluster like flowers on the branching duct tree (Fig. 1, *A* and *B*). Each lobule is drained by its own terminal duct that is connected to the duct tree. Since we learned that disease usually occurs within a given lobule and its terminal duct, it was called the terminal ductal-lobular unit.[2] Terminal ductal-lobular units are hormone-sensitive, potentially milk-producing glands that vary from 1 to 8 mm in diameter in the nonpregnant state. Most are <2 mm in diameter. Each breast contains thousands of terminal ductal-lobular units. From the intralobular part of the terminal duct, branching test-tube–like ductules arise (Fig. 1, *A*). Each terminal ductal-lobular unit can be likened to a surgeon's glove with 50 fingers. Lobules normally regress at menopause, leaving stubby small terminal ducts connected to the duct tree. However, in cancer-associated breasts, terminal ductal-lobular units often remain well developed beyond age 55.

Certain lesions formed a morphologic continuum between normal lobules and ductal carcinoma in situ when their microarchitecture (Fig. 2, *A*) and histopathologic structure (Fig. 2, *B*) were correlated. They were called atypical lobules type A.[2] Fig. 3, *A* and *B*, and 4, *A* and *B*, illustrate grade I and grade III lesions on these altered terminal ductal-lobular units. These lesions were believed to be precancerous. Statistical anal-

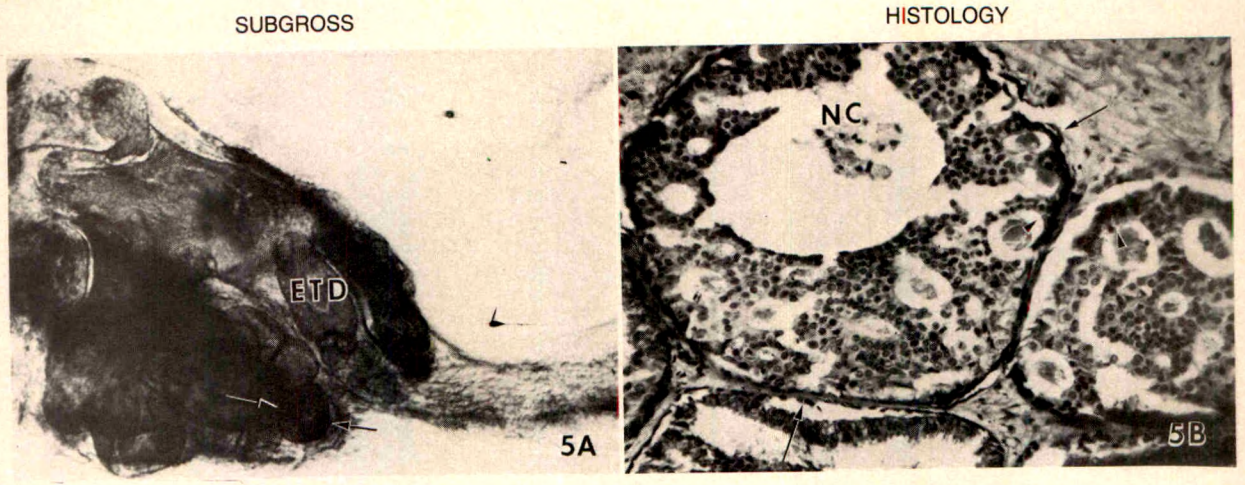


Fig. 5. *A*, ALA V (Ductal carcinoma in situ). *Arrows* point to a large cellular ductule. The *ETD* is distended with cells. (×13.) *B*, ALA V. One ductule outlined by *arrows*. It is distended with cells. *Arrowheads* point to cartwheels. Necrotic cells *(NC)* are present in the lumen. (×125.)

yses disclosed them to be cancer associated.[3] They were more abundant and of a higher degree of atypia in cancer-associated breasts.

In situ ductal carcinoma is found in terminal ductal-lobular units.[1] Multiple foci are commonly scattered throughout the breast.[2] The microarchitecture is grossly distorted into a coarsely lobulated structure, but the entire lesion still maintains the basic appearance of a terminal ductal-lobular unit with a delicate terminal duct (Fig. 5, *A*). In conventional light microscopic preparations the ductules are so big that they look like ducts (Fig. 5, *B*).

If a terminal ductal-lobular unit shows evidence of disease, it is usually of the same type and to a similar degree throughout all the ductules belonging to that terminal ductal-lobular unit. It was therefore postulated that a terminal ductal-lobular unit is of monoclonal derivation and that it originates from a growth zone, much as the way a hair bulb forms a hair.[4] In cancer-associated breasts where cell proliferation within terminal ductal-lobular units may be very active, cells pile up as plaques or as papillomas within the terminal ducts close to the lobule. Therefore it was hypothesized that the growth-zone for a given lobule is located at the interphase between the intralobular part of the terminal duct and the extralobular part of it.[4]

Comment

It had been realized many years before our research was undertaken that three-dimensional studies of breast tissue might cast new light on the pathogenesis of breast cancer; Parks[5] suggested that breast cancer originates in the terminal ductal-lobular unit.

It would be logical that the biologically active part of the breast parenchyma, the terminal ductal-lobular units, would be the site of origin of breast cancer, since they are composed of labile cells with constant cell turnover. Throughout the body it is the labile cell populations that are prone to cancerous transformation such as the cells lining the gastrointestinal tract, the respiratory tract, the genitourinary tract, the skin, and the bone marrow.

Our morphologic study indicated that the cancerous breast is diffusely afflicted with hypercellular changes in terminal ductal-lobular units, which we called atypical lobules type A. A large study was undertaken in which women who had undergone breast biopsies years before were subsequently investigated for development of breast cancer. The women with grade III and grade IV atypical lobules type A in the earlier breast biopsies developed breast cancer more frequently than expected.[6]

The fact that histologically normal-appearing terminal ductal-lobular units tend to persist beyond the menopause in women with breast cancer may be extremely important in understanding the causes of breast cancer. Do women at risk for breast cancer maintain terminal ductal-lobular units because of continual hormonal stimulation of the units or are terminal ductal-lobular units at risk endowed with more hormone receptors?

Terminal ductal-lobular units can be isolated as distinct entities after supravital staining. The cells do not appear damaged by the staining, judging from transplantation studies[7-9] or from tissue culture studies.[10] The biologic behavior of terminal ductal-lobular units should undergo extensive research so that we may gain further knowledge of the "seed" in which breast cancer starts.

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TRANSACTIONS OF THE FIFTY-THIRD ANNUAL MEETING OF THE CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

The Golden Thread

Presidential address

George D. Malkasian, Jr., M.D.
Rochester, Minnesota

In 1859 Charles Darwin published his book, *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life.* The first few chapters describe artificial selection by man as well as natural selection as a consequence of the struggle for existence. The latter, natural selection of fortuitous variations, gave a scientifically satisfactory explanation of evolution without any necessity for miraculous interposition or supernatural interference with the ordinary laws of nature. This then led to extensive studies of responses of individual organisms to their environments and of specific effects of the environment on species. A short while later Alfred Russel Wallace extended Darwin's thoughts with the observation that man, with the use of the specialized human brain, had avoided the problems that had befallen so many other species. This brain has been used to develop what we have come to accept as science with all of its good and, at times, bad prospects.

A bit over 100 years later, Eiseley[1] noted with fascination that on the great stage of evolution was a play with a strange quality about it: most of the characters had begun in generous latitudes of space and ended by being pinched out of existence in a small corner with no outlet. Biologic evolution was then defined by an endless series of specializations. For many species this specialization became the road to extinction.

It is relevant to quote Eiseley[1]:

Civilized society, confronted by innumerable inconsistencies and strains, begins to knock and vibrate like an overheated motor. The long, slow turns of world-time as the geologist has known it, or the invisibly moving hour hand of evolution perceived only yesterday by the biologist, has given way in the human realm to a fantastically speeded-up social evolution induced by industrial technology. So fast does this change progress that a growing child strives to master the sociological mores of a culture which might, compared with the face of past history, compress centuries of change into his lifetime. I myself, like many among you, was born in an age which has already perished.

In an essay on how to deal with progress in the Nuclear Age, Teller[2] stated,

One proposal has been a simple one; I have even found people who call it a logical one: Progress has led to danger; let us stop progress; if possible, let us turn the clock back . . .. As a man who has tried to be an educator, a man who has dabbled in research, I know that to learn is difficult, but I know also that to unlearn is impossible. I am furthermore quite convinced that not only can the clock not be turned back but it cannot even be stopped.

In Abraham Lincoln's words, "The dogmas of the quiet past are inadequate to the stormy present. As our case is new, so must we think anew and act anew."

For many years, health care systems have adapted to the needs of the time. What is new is the rapidity with which we are being asked to change. We can choose to try to go back to the good old days. We may elect to resist change—inviting with this the extinction of our species. Finally, as obstetricians and gynecologists, we can adapt in order to achieve new systems for delivery of health care.

We, in the medical field, have long believed in certain basic values: dedication to meeting patients' needs; dedication to social responsibility; and, above all, the Golden Thread we have established and clung to while adapting to change, dedication to high-quality medical care. Our constant attention to high quality is the very strength of health care as it exists today.

From the Department of Obstetrics and Gynecology, Mayo Clinic and Mayo Foundation.
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To analyze the challenge of change, Cooney[5] noted that the current strengths of American health care come from the existence of excellence, but there currently is in progress a revolution that is unique in terms of size, rapidity, strength, and publicness in our present health care delivery system and is distinct from the evolutionary process. The following are his major points of discussion: "(1) the results of inevitable revolutionary excesses on individuals and institutions; (2) ethics and medical-care technology; (3) cost of care and its effects on access to care; and (4) the health service and resource disproportions latently inherent in our population." To these, we should add (1) undergraduate medical education and residency training, (2) medical professional liability problems, and (3) individual attitudes and practices concerning health.

This is a list familiar to all of us and usually it is only discussed from its negative aspects. Just as the news media, with its Dickensian view, rarely reports beautiful summers or happy days, we rarely look for a positive side to these changes. I hope to point out before we conclude today that many positive things have evolved from our addressing and adapting to the demands presented by the points mentioned above, and that the Golden Thread, although stretched and at times tried to its utmost, remains intact.

The inevitable revolutionary excesses

The inevitable revolutionary excesses that were to try our ability to provide high-quality medical care began with federal action in the 1960s. It was then that we learned that federal priorities are statements, documents, speeches, etc., whereas federal policy is dollars, budgets, and budget justification. The early priorities of those years were to provide the greatest good for the greatest number. The political banner that was raised read "medical care is a right." The political priorities in 1985 still are increased access to quality care and increased knowledge of disease and its prevention. At the same time they include efforts to decrease the costs of delivering that care and of discovering those facts.

The policies of the 1960s led to annual expenditures for health care in the United States of $12 billion in 1960 and of $387 billion in 1984 (12% of the gross national product). The positive side of this expenditure is that increasingly complex procedures have become available to an increasingly larger number of people who could not afford them heretofore. The medical system evolved and was able to expand its services—physically, technically, and with appropriate well-trained personnel. The system expanded under the direction of knowledgeable health care planners who had quality care in mind. Today's policies should be implemented by similar trained personnel prepared to accomplish the forced consolidation.

In 1985 the priority for the thinking person has changed to "the greatest good for the greatest number *with the funds that are available,*" and we now debate whether all-inclusive medical care is a right or a privilege. If we stand by its being a right, then there must be free access for the entire population for all medical care. Is this not similar to the lesson we were taught by OPEC? Medical resources, like oil resources, are limited—they are not infinite as our political associates would lead their electorates to believe. Put another way, can quality care be available to all without loss of access or loss of quality? I believe the answer to both is Yes, with the proper implementation of preventive health care that can be of high quality and can strengthen the Golden Thread.

Ethics and medical care technology

Ethics and medical care technology have become increasingly intertwined. It is interesting that, in a time when we have achieved mastery over so many things in our physical environment, this mastery in itself leads to the need to involve the philosopher, the ethicist, and the clergy. We must involve these disciplines as we apply new science to our practice. There are areas in our specialty that need such input—for example, ultrasonography with its ability to diagnose fetal intrauterine problems; as a consequence of this ability, the development of intrauterine surgery; in vitro fertilization with its spin-offs of surrogate ovum transfer and surrogate ovum fertilization and transfer; cryopreservation of fertilized ova; amniocentesis for diagnosis of genetic or neural tube disorders; fetoscopy for visualization and diagnostic evaluation of the fetus; chorionic villus biopsy for evaluation of chromosomal anomalies; chemotherapy of gynecologic malignancies with agents known to carry with them the risks of future leukemias; the use of gonadotropin-releasing hormone to regulate the anterior lobe of the pituitary; the entire topic of delivery decisions made on subtle changes and interpretations of fetal monitoring strips; and the dilemma of clinical research with its goal of improved clinical outcome for various diseases.

We have accepted the science of these changes quite readily, and the techniques have been applied to our practice, adding further to the excellence and quality of care. However, the resolution of the ethical issues has lagged far behind. It is not only the physicians who are in this situation. At a recent conference I attended in Albany, New York, some of the nation's leading ethicists were present, and there was no agreement among them on any issue discussed. Neither the evolution nor the revolution of technology has been accompanied by

an advance in ethics—a major loss to our patients. It remains then for the individual clinging to the Golden Thread of quality care to provide the integrity and moral responsibility for the best application of these techniques.

Cost of care and its effects on access to care

As the costs of care become an increasingly important item, they must be continually evaluated for their effects on patient access to the Golden Thread. Reviewing National Cancer Institute grant budgets is a useful method of comparing costs of health care. It quickly becomes evident that (1) costs are not similar throughout the nation, (2) they are significantly higher in some areas than in others, and (3) they continue to increase at a rate greater than that of general inflation. The prophets of gloom would predict a headline reading "No funds available for quality care, research, teaching, or advanced technology." If we do not hear the voice of change, this headline may indeed appear. Institutions, as well as individuals, have an instinct for survival and many not-for-profit institutions are turning into corporate entities and are becoming at least partially, if not entirely, for-profit operations.

Some view the changes as tremendously creative.[3] These changes include innovative executive management that supports creativity, marketing, and total for-profit management. Philip Birnbaum, George Washington Medical Center's dean of administrative affairs, emphasized the need for new capital in solving a hospital's problems. For example, his institution's charitable contributions have decreased as a percentage of hospital income, from 21% in 1968 to 4% in 1981. In the same period, governmental contributions dropped from 23% to 12% and hospital debt as a percentage of income rose from 38% to 69%. Not-for-profit institutions do not have the same leverage to raise capital that for-profit corporations have. For example, the former can sell bonds to raise relatively small quantities of money, but the latter can sell stock, thus obtaining a greater number of dollars from the capital market. These institutions have reached innovative agreements with for-profit corporations to maintain control of teaching, research, and indigent care at or above present levels.

Those opposed to for-profit management suggest that the poor will be eliminated from consideration in these contracts. The 1984 statistics available suggest this is not the case. For example, 4.4% of the gross patient revenues of tax-paying, for-profit, investor-owned hospitals went to charity care and bad debt—a percentage exactly the same as that among private, not-for-profit institutions. This suggests that there has been no decreased access to dollars for providing care for the indigent in these institutions.

Other positive institutional innovations, although at times cumbersome, have been strict utilization reviews, sharing of expensive technologies, and specialization in services for which they are uniquely fitted.

While maintaining quality care, physicians have responded by performing more workups and operations as outpatient procedures and, when possible, avoiding weekend hospital admissions other than for emergencies.

During the past year the rate of health care inflation dropped to 9.1% from a previous high of 13.9% in 1982 and an average of 12.7% for the previous 14 years. This occurred during the first year that the system of diagnosis-related group payments was implemented.

A freeze of physicians' fees has also been implemented. But physicians have not been singled out for wage freezes; current legislative proposals are aimed at also freezing Medicaid administrative costs. This is to be achieved by limited federal administrative grants to states rather than open-ended state entitlements.

The medical care system has shown that it can adapt to the policies and priorities of federally mandated change and still provide quality care.

The health service and resource disproportions latently inherent in our population

Disproportionately expensive health care needs of small segments of our population are raising serious questions about our ability to address these needs and still provide quality care for the population at large.

Current indications are that four technologies—kidney dialysis, coronary bypass, infant intensive care, and artificial hearts—could account for $9 billion in annual expenditures. These certainly represent inequities in cost and present us with another decision: the continued use of developed technology as a criterion of high-quality care. It has been repeatedly shown, for example, that if we as obstetricians are able to maintain pregnancies in utero for longer periods when premature labor threatens, the cost of the total care for that mother and infant is less than if delivery is premature. In our area the difference between maintaining a pregnancy in utero rather than in a neonatal intensive care unit is currently about $900 a day.

Unfortunately, medicine is often compared to industry, in which technologic advance means doing things faster, more efficiently, and more cheaply. This is not true in medicine, and the advances tend to become more highly specialized rather than broadly based. However, these advances have led to increasingly more accurate diagnosis, to prolongation of life, and to cures for many diseases. These technical advances continue to reinforce our Golden Thread by producing high-quality care.

Technology as it is today, without further refinement, is consuming a larger and larger proportion of the health care dollar. As the third-party payers consider cost containment and all of its implications, it behooves us to use these advances wisely, to educate our patients as to their uses and purpose, and to educate our trainees to use them appropriately. This may allow us, rather than a third-party payer, to determine who receives what services.

The largest concern regarding disproportionate care is the increasing percentage of the population over 65 years of age. As a group, these people present specific problems that we, as gynecologists, are trained to care for and to do it better than some other specialists—osteoporosis prevention, problems associated with pelvic relaxation, and preventive observation for reproductive organ malignancies, to name but a few.

Undergraduate medical education and residency training

The Golden Thread has remained evident in the arena of undergraduate medical education and residency training. But through the years, social and political pressures have led to a demand for a greater number of physicians, a greater number of primary-care physicians, and a change in distribution of physicians to provide better care to a greater number of people.

In 1965 there were approximately 292,000 physicians in the United States, and there was a perception that more were needed. The profession and its schools responded, so that by 1982 there were about 502,000 physicians. Indeed, the response to the perceived problem was so positive that the same forces that initiated the demand now state there are 50,000 too many physicians at the present time. Despite the stated excess, new loan guarantees are available to 12,500 students and revolving loan funds to 38,500 others.

The federal government has defined the primary-care specialties as internal medicine, pediatrics, and family medicine. On the basis of this definition, the number of primary-care specialists has risen in those same years from approximately 126,000 to 174,000. Furthermore, a 1980 Rand Corporation study[4] has shown a significant change in the distribution of physicians from the metropolitan to the nonmetropolitan areas, so that smaller and smaller towns are acquiring Board-certified specialists who previously were unavailable to them locally.

It is currently stated in H.R. 2699 that it is the "explicit congressional policy" to provide for the training of increased numbers of physicians in primary care. There are currently 15,800 Board-certified obstetrician/gynecologists, and 4800 residents are in training. The number entering training has stabilized at 1200 per year and has remained stable since about 1978. The number of training programs has stabilized at approximately 300 in the United States, but this is a decrease from a high of 483 in 1963. A bill currently in Congress, S. 1210, would, among other things, establish a National Council on Graduate Medical Education which would be responsible for allocating quotas to primary-care specialist programs, perhaps at the expense of other types of specialists. This does indeed address quantity and perceived needs and services, but it does not recognize that the best quality care is provided by completely educated physicians.

Our specialty has addressed the issue of quality training by development of increasingly rigid standards for training programs, continued monitoring of the product of these programs, recertification, and provision for easy access to continuing education of high quality—that is, continuing reinforcement of the Golden Thread.

Medical professional liability problems

The heaviest strain on the Golden Thread has come as a result of medical professional liability problems. In 1975 when one in 20 practicing physicians was being sued, a crisis appeared in regard to liability premiums. Like many crises, some immediate actions slowed the increase in costs and in number of suits but did not stop the trend. Today, one in five practitioners is being sued, and the costs are escalating astronomically.

Halley[5] summarized the situation well:

> Today we do know more about the problem, but reasonable minds still differ on its precise definition. It is not primarily a problem of substandard health care practices, solvable through risk management or disciplinary action against providers. It is not primarily a controversy between physicians and trial attorneys, or between health care providers generally and the legal profession, although reports and news media coverage of events may convey this interpretation. It is not primarily a problem of the insurance industry, manifested by rising premiums, although this sector is certainly involved, as are the economy, state legislatures, state regulatory agencies, and the federal government. It *is* primarily, however, a problem of patient injuries, real or imagined, arising out of or in the course of health care delivery, and at times resulting from health care providers' negligence (Report of the Secretary's Commission on Medical Malpractice. Washington DC; Dept. HEW, January 16, 1973). It is a problem of personal injury to patients in the environment of high technology–modern health care, multiple treatment modalities and drugs, an astronomical number of decisions or individual judgments for delivery or non-delivery of care, and the occurrence of sub-optimal or bad results or treatment failures, sometimes in patients who formerly would not have survived.

The tort system, which was based on negligence and compensation for it, was expected to influence behavior

by inflicting financial loss. However, the emergence of liability insurance spreads this cost, and the impact has been diffused. The system further places demands on the physician for 100% good results and absolute safety. It also assumes it can identify the one at fault, provide a fair trial by peers, and influence behavior by financial impact if a culprit is found guilty. Now, with the application of strict liability by many states, this is no longer a functioning system. For example, in some states even though one may be judged to be only 1% or 2% at fault, if the one responsible for the rest cannot pay the entire judgment, it falls on the minor loser. The tort system is neither prompt nor efficient, and it does not provide for most of the money to go to the injured party.

Solutions proffered include (1) medical adversity insurance; (2) some form of no-fault insurance; (3) programs like workman's compensation; and (4) extensions of legislation such as exists in California with a sliding scale for attorney's contingency fees, a $250,000 limit on noneconomic (pain and suffering) awards, consideration of a claimant's other sources of compensation for injury, and periodic payment for large awards.

As the litigious attitude has expanded in our society, several other major groups—industry, the insurance business, and government—have been affected. With government now affected, the taxpayer has become the new "deep pocket," and people are becoming more aware of the overall problem.

Litigation has caused our Golden Thread to become frayed and strained to its utmost, but I sincerely believe it has not lost its continuity. Several positive elements have emerged from this as part of today's medical practice. There is increased documentation and detailing of procedures, events, and results. There is more informational dialogue with patients. Consultative services are better utilized. There is better adherence to current standard practices. There is more attention paid to better postgraduate education, licensure regulation, and hospital staff appointments. The American College of Obstetricians and Gynecologists has undertaken a pilot study with the sole purpose of evaluating risk factors within a hospital setting, and it is now training physicians to be involved in these systems of evaluation. All of the above do indeed add to costs; but as we are aware, fine-tuning a system generally costs more than the initial system, and the society in which we live already pays for it in many areas of government and industry.

Individual attitudes and practices concerning health

The Golden Thread has been spun by the medical community and sought after by the population at large with little concern on their part as to how they, as individuals, might improve their own health. American industry has paid a heavy bill for medical care for its employees, and these employees in turn have used the system heavily. Because of significant costs, industry now wants its employees to know there are choices that they can make. Health promotion and preventive efforts are being undertaken to change individual attitudes and health care practices. These include programs on smoking cessation, drug and alcohol abuse, and nutrition.

These are areas in which we, as physicians, have been involved to some degree in the past. It should come as no surprise that with the government priority on preventive medicine we will be asked to do more of these things. We face increasing competition as providers of this information. These programs fall into the catch-all of "continuous care," and as we are all aware, there is no generally accepted definition of the continuous-care provider.

Our Golden Thread has led us in the past to be involved in teaching patients on a one-on-one basis and somewhat on a group basis. Some medical societies now have undertaken the changing of attitudes on a mass-media basis with sound, useful information of a complete, well-founded nature, not an advocate's narrow viewpoint.

Conclusion

"The great thing about this world is not so much where we are but where we are going," stated Oliver Wendell Holmes.

Years ago, physicians worried about identifying disease processes and seeing their patients quietly and as comfortably as possible through the end of the natural process. We have responded to the scientific findings that allow us mastery over our own environment and improved patient care. We now find ourselves with new problems that are somewhat of our own making from our past successes. We all have shared in medicine's excellence and in creating better quality in medical practice for our patients. Now, with a clear awareness of change and adaptation as a way of life, we have great responsibility to look beyond 1985 to longer-term goals that will allow future generations access to the Golden Thread.

All of us in this organization are dedicated to the continuous spinning of our Golden Thread—high-quality care in obstetrics and gynecology. Over the years we all have been leaders in adapting to the pressures for change and are in positions—locally, statewide, and nationally—to ensure that these changes follow and strengthen our Golden Thread. Lest we feel that the pressures of change are peculiar to us in this age, let me end with a quote from an Italian statesman who urged his countrymen to change and to adapt to their times and not rely on past glories. In 1513 Machiavelli wrote in *The Prince*, "It must be remembered that there

is nothing more difficult to plan, more doubtful of success, nor more dangerous to manage than the creation of a new system. For the initiation has the enmity of all who would profit by the preservation of the old institution and merely lukewarm defenders in those who would gain by the new one."

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Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay

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Peritoneal fluid from 35 women with endometriosis and from 34 control women was aspirated at laparoscopy and analyzed. No differences in prostanoid levels were found. The peritoneal fluid volume, macrophage concentration, macrophage content, and content of activated macrophages as measured by acid phosphatase staining were all significantly elevated in the endometriosis patients. The macrophages were incubated and the medium was added to the zona-free hamster egg sperm penetration assay. This medium caused a significant decrease in the percentage of ova penetrated in this assay. It is postulated that one of the mechanisms of infertility in women with endometriosis may involve the increased number of activated macrophages and their ability to interfere with sperm-egg interaction. (AM J OBSTET GYNECOL 1986;154:1290-9.)

Key words: Peritoneal fluid, endometriosis, macrophages

It is generally accepted that women with endometriosis are less fertile. In advanced disease tubal occlusion and/or peritubal adhesions caused by endometriosis may interfere mechanically with ovulation, ovum pick-up, and ovum transport. However, in mild endometriosis the mechanisms responsible for infertility are not known.

Drake et al.[1] found that patients with endometriosis had significantly greater amounts of peritoneal fluid compared with those of disease-free women. They also showed that the peritoneal fluid of patients with mild endometriosis contained significantly higher levels of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ when compared with the fluid obtained from patients without evidence of the disease. These substances are stable metabolites of thromboxane A_2 and prostacyclin, respectively. They postulate that these products or their precursors could act on smooth muscle and interfere with tubal function. This would explain a mechanism by which mild endometriosis causes infertility. They suggested that mild endometriosis associated with infertility could be treated by regulation of prostaglandin synthetase activity.

Rock et al.[2] found that endometriosis was not associated with an increase in peritoneal fluid volume. They also analyzed the peritoneal fluid concentrations of several prostanoids in women with endometriosis and in women without evidence of the disease. They found

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that the levels of prostaglandin E_2, prostaglandin $F_{2\alpha}$, 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ (a stable product of prostaglandin $F_{2\alpha}$), and thromboxane B_2 were not significantly different in the two groups when measured during the proliferative phase of the cycle. These results were confirmed by Sgarlata et al.[3] and by Dawood et al.[4] Interestingly however, in a more recent paper, Badawy et al.[5] reported a significant difference in the levels of prostaglandin $F_{2\alpha}$ and prostaglandin E_2 in the peritoneal fluid in these two groups of patients.

Haney et al.[6] have found increased peritoneal fluid volume and increased numbers of macrophages in the peritoneal fluid of patients with infertility and endometriosis when compared with those of both fertile women and infertile women without endometriosis. They speculated that these inflammatory cells may act as antifertility effector leukocytes in the microenvironment within the pelvic cavity. Similar cell-mediated effects have been demonstrated in individuals wearing intrauterine contraceptive devices.[7] Additional work from their laboratory[8] has indicated that phagocytosis of sperm by these macrophages may indeed be a mechanism of infertility in patients with endometriosis.

Halme et al.[9] have found that mild endometriosis is associated with activation of pelvic macrophages as measured by acid phosphatase staining. They suggested that this might be a factor in the associated infertility since these activated macrophages could either phagocytose gametes or release active compounds into the surrounding fluid. They did not find a difference in prostaglandin E_2 or $F_{2\alpha}$ content or in macrophage concentration of the peritoneal fluid.

Halme and Hall[10] have shown that peritoneal fluid from patients with endometriosis did not affect human sperm penetration of zona-free hamster ova. In their study, however, all cells were removed from the fluid before it was tested, and the fluid was heat inactivated.

The purpose of this study is fourfold: (1) to compare the peritoneal fluid volume and prostanoid content in patients with endometriosis with those in women without the disease, (2) to measure macrophage concentration and macrophage activation in patients with and without endometriosis, (3) to determine if peritoneal macrophages release active substances capable of affecting sperm penetrating capacity, and (4) to investigate the possibility of altering these peritoneal fluid constituents by administering ibuprofen, a nonsteroidal antiprostaglandin agent.

Material and methods

Peritoneal fluid was aspirated from 69 women undergoing laparoscopy for evaluation of infertility or for elective sterilization. Thirty-five women were found to have endometriosis. Of these, two had Stage III disease, eight had Stage II, and the remainder had Stage I endometriosis, according to the American Fertility Society classification. Thirty-four women were disease free. Of this latter group nine women received 400 mg of ibuprofen four times a day for 3 days immediately before laparoscopy. The final dose was given at midnight before the procedure. This regimen is effective for treatment of dysmenorrhea.[11] Four of the women with endometriosis received ibuprofen.

At laparoscopy all peritoneal fluid was removed from the anterior and posterior cul-de-sac with a multiport aspirating cannula. The volume was recorded and the fluid was immediately placed into 15 ml polystyrene centrifuge tubes containing 100 U of heparin and transported on ice to the laboratory. The day of the menstrual cycle was recorded. Endometrial biopsy was also used to confirm ovulation.

An aliquot of the fluid was centrifuged at 800 × g for 10 minutes. The supernatant was frozen at −80° C and stored for future analysis for thromboxane B_2, 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$, and prostaglandin E.

Three milliliters of the remaining fluid was diluted 1 : 1 with cold phosphate-buffered saline, gently layered over 3 ml of ficoll-diatrizoate in a polystyrene centrifuge tube, and centrifuged at 420 × g for 30 minutes. The interphase cells were then centrifuged at 400 × g for 10 minutes at 4° C and the pellet was resuspended in 3 ml of freshly prepared Biggers, Whitten, and Wittingham (BWW) medium containing 3 mg of human serum albumin per milliliter. This same batch of medium was to be used for the spermatozoa penetration assay the next day. The cells were then counted in a hemacytometer.

An additional milliliter of the modified BWW medium was added and the cells were washed by mixing. The specimen was then centrifuged at 200 × g for 10 minutes at 4° C. The supernatant was discarded and the pellet was resuspended in the modified BWW media to a concentration of 1.5×10^6 cells per milliliter.

One milliliter of this suspension was then placed into a 35 mm plastic culture dish and incubated overnight at 37° C. Studies with the use of 0.4% trypan blue dye revealed no significant difference in cell viability before and after the incubation period.

The remaining suspension was diluted to 0.4×10^6 cells per milliliter. With a pasteur pipette, four drops of this suspension was placed into each of four chambers of a cytocentrifuge and cytospin slides were prepared. Two slides were stained with Giemsa stain and used to determine the percentage of macrophages. Haney et al.[6] have shown that this technique gives results within 5% of those obtained with nonspecific esterase staining. The two remaining slides were stained for acid

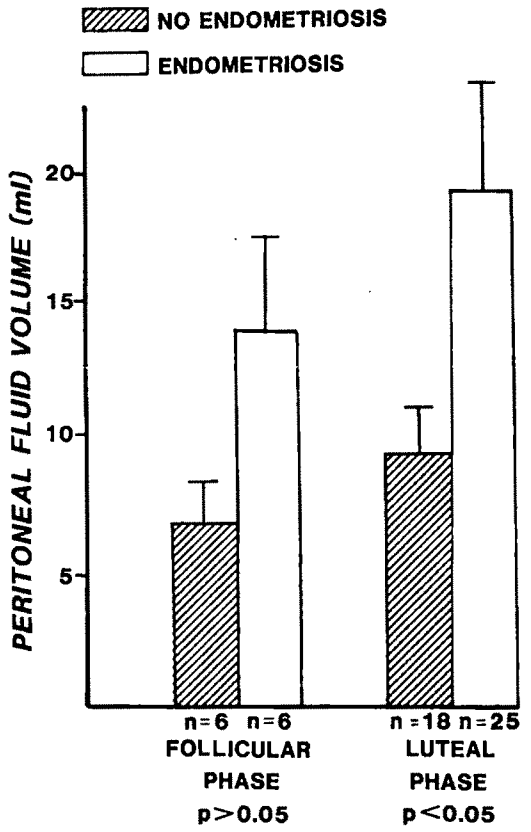


Fig. 1. Peritoneal fluid volume. *Open bars* indicate mean volume in endometriosis patients. *Hatched bars* indicate mean volume in patients without endometriosis. *Vertical lines* indicate SEM.

phosphatase activity by the method of Burstone[12] to determine macrophage activity. One thousand cells were counted for each patient.

After the overnight incubation the medium containing the macrophages was centrifuged at 800 × g for 10 minutes. It was then added to the spermatozoa penetration assay to determine if the supernatant contained any active substances that were released or secreted by the macrophages that were capable of affecting sperm penetration.

The spermatozoa penetration assay technique used in our laboratory[13] is a modification of that of Rogers et al.[14] Briefly, 0.5 × 10^6 motile spermatozoa from a donor were added to two separate insemination drops. One control drop contained 200 μl of modified BWW media and the other contained 160 μl of this medium plus 40 μl of the modified BWW medium preincubated for 18 hours with macrophages. Zona-free hamster eggs were then added to each drop and allowed to incubate for 3 hours.

Slides were made by a standard squash technique on paraffin-prepared slides. These were then "quick fixed" in glutaraldehyde and allowed to fix overnight in formalin. Fixed slides were dehydrated in ethanol, stained with acetolacmoid, and scored for penetrations under a phase-contrast microscope (40 × dry objective). The percentage of penetration (number of ova penetrated per number of ova) and the penetration index (number of spermatozoa penetrating ova per number of ova penetrated) were both determined.

Table I. Peritoneal fluid prostanoids in women with and without endometriosis

	Endometriosis (n = 5)	*No endometriosis (n = 5)*	*p*
Concentration (ng/ml)			
TxB_2	0.068 ± 0.019	0.051 ± 0.009	NS
PGFM	0.407 ± 0.188	0.237 ± 0.020	NS
6-Keto-$PGF_{1\alpha}$	0.442 ± 0.117	0.340 ± 0.009	NS
PGE	0.552 ± 0.086	0.588 ± 0.018	NS
Total content (ng)			
TxB_2	1.48 ± 0.55	0.84 ± 0.13	NS
PGFM	6.63 ± 1.95	3.66 ± 0.66	NS
6-Keto-$PGF_{1\alpha}$	9.78 ± 3.43	5.03 ± 0.72	NS
PGE	8.57 ± 1.05	11.11 ± 2.47	NS

Values are mean ± SEM. TxB_2 = Thromboxane B_2; PGFM = 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$; 6-keto-$PGF_{1\alpha}$ = 6-keto-prostaglandin $F_{1\alpha}$; PGE = prostaglandin E.

Prostanoids were determined by radioimmunoassay. The technique has been described.[15] Briefly it consisted of solvent extraction, purification on a silicic acid chromatography column, and radioimmunoassay with the use of antibodies raised against the prostanoids of interest.*

The two-tailed *t* test and multiple analysis of variance were used for statistical evaluation where appropriate.

Results

The results of the peritoneal fluid volume analysis are seen in Fig. 1. In the follicular phase of the cycle

*The 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ antiserum (560-JCC-140D, The Upjohn Co., Kalamazoo, Michigan) did cross react with the prostaglandins as follows: 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, 100%; 15-keto-prostaglandin $F_{2\alpha}$, 20%; and the C16 metabolite of prostaglandin $F_{2\alpha}$, 13,14-dihydro-prostaglandin $F_{2\alpha}$, and prostaglandin $F_{2\alpha}$, <1%. Cross-reactivity for the thromboxane B_2 antiserum (SG1003, Seragen, Boston, Massachusetts) was: thromboxane B_2, 100%; prostaglandin E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$, <0.1%. Prostaglandin E antiserum (1001, Seragen) cross-reactivity was: prostaglandin E_1, 100%; prostaglandin E_2, 100%; prostaglandin A_1, 3%; prostaglandin A_2, 6%; 6-keto-prostaglandin E_1, 1%; prostaglandin $F_{2\alpha}$, 1.3%; prostaglandin $F_{1\alpha}$, 1%; 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2, <1%. Cross-reactivity for the 6-keto-prostaglandin $F_{1\alpha}$ antiserum (SG 1004, Seragen) was as follows: 6-keto-prostaglandin $F_{1\alpha}$, 100%; prostaglandin $F_{1\alpha}$, 7.8%; 6-keto-prostaglandin E_1, 6.8%; prostaglandin $F_{2\alpha}$, 2.2%; prostaglandin E_1, 0.7%; prostaglandin E_2, 0.6%; thromboxane B_2, 15-keto-prostaglandin $F_{2\alpha}$, and 15-keto-prostaglandin E_2, <0.01%.

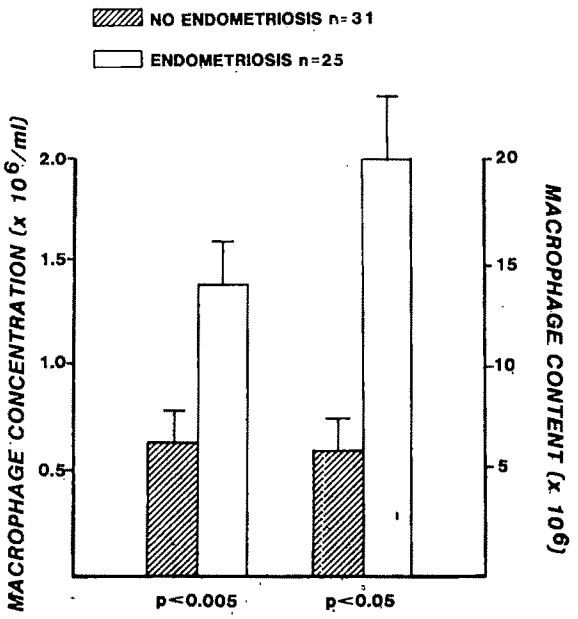


Fig. 2. Mean peritoneal fluid macrophage concentration and content in endometriosis patients *(open bars)* and patients without endometriosis *(hatched bars)*. *Vertical lines* indicate SEM.

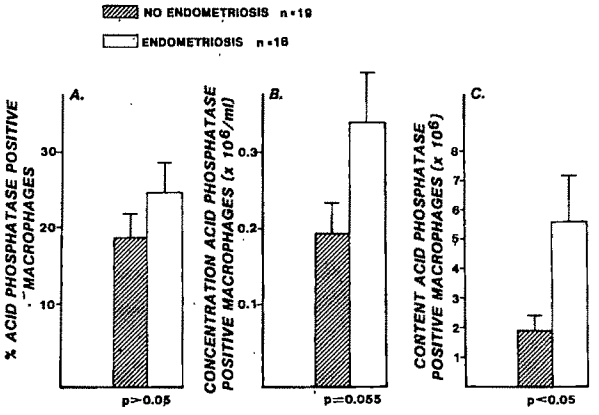


Fig. 3. Mean peritoneal fluid acid phosphatase–positive macrophage percentage *(A)*, concentration *(B)*, and content *(C)*. *Open bars* indicate values in endometriosis patients. *Hatched bars* indicate values in patients without endometriosis. *Vertical lines* indicate SEM.

the peritoneal fluid volume in milliliters found in the pelvic cavity of women with endometriosis, 13.8 ± 3.6 (mean + SEM), was larger than but not significantly different from that obtained from disease-free women, 6.7 ± 1.4. In the luteal phase, however, a significantly larger volume of peritoneal fluid was found in women with endometriosis, 19.2 ± 4.0 ml, than in women without this disease, 9.3 ± 1.5 ml ($p < 0.05$). Ibuprofen had no significant effect on peritoneal fluid volume.

Cul-de-sac prostanoids (15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$, thromboxane B_2, 6-keto-prostaglandin $F_{1\alpha}$, and PGE) in women with endometriosis were not significantly different from those of the control group. This remained true for both prostanoid concentration and total content (Table I). During the course of this study other investigators[3, 4, 9] confirmed these results, which were consistent with the earlier report of Rock et al.[2] Because of this information, in combination with our further findings that neither ibuprofen nor phase of the menstrual cycle affected prostanoid levels in women without endometriosis, we elected to divert our efforts toward analysis of peritoneal fluid macrophages.

Women without endometriosis had a significantly lower concentration of macrophages in the peritoneal fluid (0.65 ± 0.10 × 10^6 cells per milliliter, mean ± SEM) when compared with that of women with this disease (1.39 ± 0.20 × 10^6 cells per milliliter, $p < 0.005$). The total macrophage content of the peritoneal fluid in patients without endometriosis was 5.75 ± 1.39 × 10^6 cells (mean ± SEM), which was also significantly lower than 19.88 ± 3.31 × 10^6 cells, found in women with endometriosis ($p < 0.01$) (Fig. 2).

The phase of the menstrual cycle was found to affect the macrophage concentration. Women in the follicular phase (not including the menstrual phase) had a mean macrophage concentration (±SEM) of 1.64 ± 0.20 × 10^6 cells per milliliter, higher than 0.87 ± 0.11 × 10^6 cells per milliliter found in luteal phase fluid (0.006 = $p < 0.01$). Ibuprofen did not affect macrophage concentration or content.

The mean percentage (±SEM) of positive macrophages for acid phosphatase in the peritoneal fluid of women without endometriosis was 18.26 ± 3.39 (Fig. 3). This value was higher in the peritoneal fluid of endometriosis patients (24.54% ± 3.90%); however, the difference was not significant.

The mean concentration (±SEM) of acid phosphatase–positive macrophages in the peritoneal fluid of women without endometriosis (Fig. 3) was lower (0.19 ± 0.04 × 10^6 cells per milliliter) than in patients with endometriosis (0.34 ± 0.07 × 10^6 cells per milliliter). In this case the difference approached significance ($p = 0.055$) (Fig. 3).

Finally, the number of acid phosphatase–positive macrophages in the peritoneal fluid of women without endometriosis (1.89 ± 0.57 × 10^6, mean ± SEM), as seen in Fig. 3, was significantly lower than the number found in endometriosis patients (5.64 ± 1.60 × 10^6, $p < 0.05$).

Neither the phase of the menstrual cycle nor the administration of ibuprofen had a significant effect on activated macrophages as measured by acid phosphatase staining.

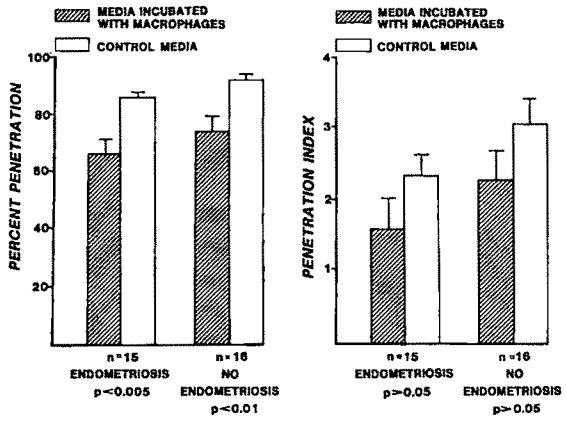


Fig. 4. Mean percentage of penetration obtained with medium incubated with peritoneal macrophages from patients with and without endometriosis. *Open bars* indicate control values. *Hatched bars* indicate patient values. *Vertical lines* indicate SEM.

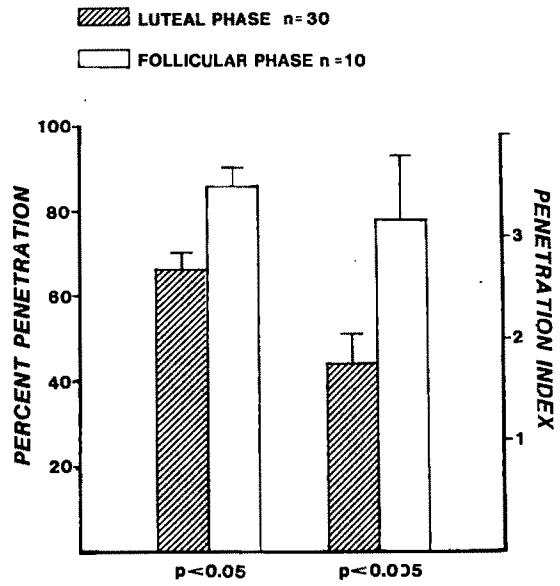


Fig. 5. Mean percentage of penetration and penetration index obtained with medium incubated with peritoneal macrophages obtained during either the luteal *(hatched bars)* or follicular *(open bars)* phase of the menstrual cycle. *Vertical lines* indicate SEM.

Medium in which peritoneal macrophages had incubated was found to have a significant effect on the number of ova penetrated in the spermatozoa penetration assay in both endometriosis patients and women without the disease (Fig. 4). The mean percentage of penetration (±SEM) in assays where the medium had been incubated with peritoneal fluid macrophages from endometriosis patients was 66.33 ± 5.94 whereas control medium produced a mean (±SEM) percentage of penetration of 85.66 ± 2.17 ($p < 0.005$) in the same assays. Medium that had incubated with macrophages from patients without endometriosis resulted in a mean (±SEM) percentage of penetration of 73.31 ± 5.98. Control medium in these assays produced a value of 92.12 ± 2.12 ($p < 0.01$).

Although medium that had incubated with peritoneal macrophages had a tendency to lower the mean (±SEM) penetration index both in endometriosis patients (1.59 ± 0.45 versus 2.31 ± 0.33 for control women) and in disease-free women (2.27 ± 0.40 versus 3.06 ± 0.035 for control women), this difference was not significant.

The phase of the menstrual cycle was found to have a significant effect on the percentage of penetration and apparently on penetration index (Fig. 5). The mean percentage of penetration (±SEM) produced by medium that was incubated with peritoneal macrophages recovered during the follicular phase of the cycle was 85.00 ± 4.78, which was significantly higher than that resulting from medium that had been incubated with peritoneal macrophages aspirated during the luteal phase, 65.60 ± 3.94 ($p < 0.05$). The mean penetration index (±SEM) produced by follicular macrophage–incubated medium was 3.09 ± 0.66, whereas the corresponding result for luteal macrophage medium was 1.71 ± 0.27 ($p < 0.005$).

The penetration index was also apparently significantly affected by ibuprofen. The mean penetration index produced by medium that had been incubated with peritnoneal macrophages recovered from women who received ibuprofen was significantly higher than that seen with medium that had been incubated with peritoneal macrophages from women who did not receive this nonsteroidal anti-inflammatory agent ($p < 0.05$). However, a two-way interaction between ibuprofen status and phase of the menstrual cycle approaching significance ($p = 0.051$) was found for the penetration index variable, indicating that the apparent effects of ibuprofen and phase of the menstrual cycle on penetration index may be the result of confounding variables.

With the use of discriminant analysis a mathematical model was developed in an attempt to predict which patients would have endometriosis on the basis of the results of selected variables. The variable selected by the model to be most predictive of endometriosis was the macrophage concentration followed by the number of acid phosphatase–positive cells, and the macrophage number in that order. Twenty-four patients without endometriosis and 21 patients with this disease had the necessary data to be entered into the model. Of the former group 22 patients (91.7%) were correctly predicted to be endometriosis free whereas two patients (8.3%) were predicted to have the disease. Of the patients with endometriosis 13 (61.9%) were correctly predicted to have the disease by this model whereas eight

patients (38.1%) were predicted to be disease free. Overall 77.78% of cases were correctly classified by this mathematical model.

Comment

We have found no difference in the peritoneal fluid volume between women with and without endometriosis in the follicular phase of the menstrual cycle. In the luteal phase, however, women with endometriosis had a significantly larger volume of peritoneal fluid. When these two groups were combined the difference remained significant. These findings are consistent with those of Drake et al.[1] and Haney et al.[6] (increased peritoneal fluid volume in endometriosis patients) and with those of Rock et al.[2] (no difference in peritoneal fluid volume in the follicular phase).

Our prostanoid data are consistent with those of Rock et al.,[2] Sgarlata et al.,[3] and Dawood et al.,[4] indicating that peritoneal fluid prostanoids are probably not elevated in women with endometriosis. The finding that prostanoid levels, in our study and others,[2-4] were much lower than those reported by Drake et al.[1] and by Badawy et al.[5] suggests that substances other than the specific prostanoid may have been measured.

The increased level of macrophages found in the peritoneal fluid of women with endometriosis suggests that such macrophages may play a role in the infertility of these patients. The increased number of activated macrophages in patients with endometriosis, consistent with the data of Halme et al.,[9] also is supportive of such a concept.

These macrophages may phagocytose sperm as demonstrated by Muscato et al.[8]

Macrophages may also release substances into the pelvic environment that can inhibit sperm-egg interaction.

We have found that medium incubated with peritoneal macrophages caused a significant decrease in the number of zona-free hamster eggs penetrated by sperm in our spermatozoa penetration assay. Macrophages from endometriosis patients, however, were no more effective at decreasing the percentage of penetration than macrophages from disease-free women. Thus, if macrophages play a role in the infertility of women with endometriosis, they do so because of their increased number, not because of any particular property of the macrophages found in women with endometriosis. In our experimental design, macrophages from endometriosis patients as well as from disease-free women were brought to the same concentration before incubation. Our spermatozoa penetration assay results therefore do not reflect the larger concentration of macrophages found in endometriosis patients. Macrophages may release substances into the pelvic environment that are capable of decreasing sperm penetration; infertility in endometriosis patients may be due in part to the larger number of macrophages in these women. These substances, as yet undetermined, are heat labile since heat-inactivated peritoneal fluid has no effect on the penetration of hamster ova by sperm.[10] Additional studies are currently being undertaken in our laboratory to further characterize this effect.

Why medium incubated with macrophages obtained during the luteal phase was more effective in decreasing sperm penetration parameters than that incubated with macrophages from the follicular phase is unclear. A similar study performed in the periovulatory period would have more clinical relevance.

At the onset of the study we postulated that peritoneal fluid prostanoids and macrophages might be causally related to the infertility in endometriosis. We therefore evaluated the ability of ibuprofen (a prostaglandin synthetase inhibitor and nonsteroidal anti-inflammatory agent) to alter prostaglandins or inflammatory cells in the peritoneal fluid.

Surprisingly the only parameter among those evaluated significantly affected by ibuprofen was the penetration index. Ibuprofen seemed to lessen the ability of macrophages to release factors that decrease sperm penetration. The number of patients who received ibuprofen and were found to have endometriosis, however, was small ($n = 4$) and further work is needed to confirm this finding. If the finding is confirmed, a therapeutic role for ibuprofen in endometriosis-related infertility may be considered.

The inability of ibuprofen to affect peritoneal fluid prostaglandin levels and other macrophage parameters in our study may be due to the dosage schedule. The dosage used is effective in treating dysmenorrhea, indicating its effect on endometrial prostaglandins. However, Jones et al.[16] have demonstrated that prostaglandin content of some tissues can be affected more readily than others by antiprostaglandin administration. In peritoneal fluid of the rabbit, prostaglandin values are relatively insensitive to indomethacin administration when compared with kidney or blastocyst prostaglandin values.

The development of a mathematical model to predict endometriosis could potentially become a tool in anticipating which patients may have disease that is not evident at the time of laparoscopy. Although the number is small, follow-up of the women who were predicted by the model to have endometriosis, yet who had no visible disease at the time of laparoscopy, should prove interesting. If these women are found to develop endometriosis, our model may prove to be a valuable instrument to determine which patients may have latent or occult disease.

In conclusion, we have demonstrated that patients with endometriosis have more peritoneal fluid that con-

tains more macrophages when compared with fluid in women without this disease. Endometriosis patients also have a larger number of activated macrophages in the peritoneal fluid. In addition these macrophages appear to be releasing compounds that are capable of decreasing sperm penetration. Whether ibuprofen may be able to reverse this phenomenon is not clear.

We gratefully acknowledge the assistance of Dr. Marija Norusis in performing the statistical analysis, and the technical assistance of Ms. Hsui Tseng and Mr. Dot Chin. We also thank the Clinical Cytology Department at Michael Reese Hospital for allowing us to use their facilities.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Hassan Amirikia, Detroit, Michigan. The association of endometriosis and infertility is well known[1]; however, the exact mechanisms involved remain unclear and are as controversial as the etiology of the endometriosis. In addition, even when women with endometriosis do conceive, recent studies indicate a significant increase in the incidence of first trimester spontaneous abortions in untreated patients.[2]

To identify the factor(s) involved in the pathophysiology of infertility related to endometriosis, many studies have been performed during the last decade. Their authors have concluded that peritoneal changes in endometriosis may lead to infertility in a variety of ways: (1) Increased peritoneal fluid prostaglandins may cause tuboovarian or uterine dysfunction and interfere with fertilization and/or implantation. (2) Luteinization may occur without ovluation. Donnez et al.[3] observed fewer ovulation ostia in ovaries of patients with endometriosis compared with those in normal ovulatory women and ostia that were comparable with those of patients with the luteinized unruptured follicle syndrome. (3) The increased number and activity of the peritoneal fluid macrophages may interfere with fertilization by an increase in phospholipases, which act on cell membrane phospholipids to produce arachnoid acid used in the synthesis of prostaglandin at the site of the reaction,[4] or by an increase in phagocytosis of the spermatozoa.[5]

Dr. Chacho and associates, in their study, concluded that patients with endometriosis have more peritoneal fluid that contains increased macrophages. Although the concentration and percentage of active macrophages were not different, patients with endometriosis have a larger number of activated macrophages compared with those in normal women. In addition, they proposed a very interesting concept that the macrophages may be releasing compounds that could interfere with fertilization as demonstrated by decreasing sperm penetration into hamster eggs. Ibuprofen seemed to lessen the ability of macrophages to release this factor although the number of women receiving this antiprostaglandin agent was small. This is a very interesting study and we hope the authors pursue their work further to identify this factor, which could help us to understand the exact mechanism of endometriosis and also, in the future, direct us to develop a new method of contraception.

There are, however, some questions about this study. Dr. Chacho and associates have reviewed 69 patients who underwent laparoscopic procedures for infertility

or sterilization. They found 35 patients with endometriosis and 34 without. It is not clear how many of these 35 patients with endometriosis were fertile and had just had the laparoscopic procedures for the purpose of sterilization; what were their findings in the peritoneal fluid in this group of patients? The authors state that the amount of peritoneal fluid was higher in the luteal phase than in the follicular phase, yet only six women in each group were studied during the follicular phase; 18 of 25 were in the luteal phase. Is six cases a large enough sample to be entirely confident of this statement? In both follicular and luteal phases, the mean peritoneal fluid volume of women with endometriosis was twice that of those without endometriosis. If the number of women in the follicular phase were greater, both probably would have been significant. Prostanoid concentrations are reported for only five of 35 in the first group and five of 34 in the second group of women; how do the authors know that these five are representative of the other 30 and 29 women? If they had done more measurements, they might have seen different results. In addition, the collection of samples and the performance of radioimmunoassays for prostanoids are fraught with potential problems.[6] Are the authors certain that their methods have overcome these problems? They cite a 1985 article as validation. In order to study macrophage secretions, macrophages were incubated overnight with medium and cell viability was checked the next day by trypan blue exclusion. However, in the presence of the exogenous protein (human serum albumin) this method can give inaccurately high values.[7] If the macrophages were dying, they could have released factors not normally present in intraperitoneal fluid that could reduce the percentage of penetration of sperm into the hamster egg. These factors may not represent normal cell function. The authors should have examined cell viability by another method, such as phase contrast refraction.

These authors have constructed a model from their data and then applied it to their data. The fact that they obtained a 77.8% correct classification is surprising; it should have been closer to 100% if the model were properly constructed. They need to use the model on another group of women to test its "goodness of fit."

The critiques regarding sample size applied to Fig. 1 presented by the authors also can be applied to Fig. 5 (percentage of penetration and penetration index). The number of follicular phase samples was one third that of the luteal phase. With higher numbers, the significance level could be different.

Although there were 35 and 34 subjects in Groups 1 and 2, respectively, the highest numbers in the figures are 25 and 31. There is a lack of data for some parts of this study, and if data from the whole group are not reported, how can one say the population with endometriosis is properly represented? This is a promising pilot study if the methodologic questions raised can be answered adequately.

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DR. L. RUSSELL MALINAK, Houston, Texas. Dr. Chacho and colleagues have undertaken an extensive research project directed at the answer to one of the most elusive problems in gynecology, namely, the etiology of infertility in endometriosis patients when anatomic distortion of the internal genitalia has not occurred.

There are several questions that must be clarified.

First, regarding patient selection, it is stated that laparoscopy was performed for evaluation of infertility and for elective sterilization and that 35 patients had endometriosis and 34 were disease free. It is not stated, however, how many patients in each group had endometriosis, a fact important in interpreting these data. We have all seen endometriosis at the time of laparoscopy for tubal sterilization; such a finding, if present or not observed at laparoscopy for tubal sterilization, would bias the control group. Also, findings such as pelvic adhesions, leiomyomas, or other pelvic abnormalities that might alter peritoneal fluid and macrophage physiology are not mentioned in either group. Patient demographics and operative findings are thus incomplete. For example, if some patients in the sterilization group had intrauterine contraceptive devices in situ at the time of the laparoscopic procedure, macrophage changes might be observed, which circumstance would eliminate that patient as a control.

Regarding study design, it is of concern that the authors studied peritoneal fluid only in the proliferative phase and the luteal phase. The dynamism of each phase of the cycle demands that biochemical and cell population studies be evaluated at several times during each phase. Contrast in your mind's eye the observable differences of peritoneal fluid at laparoscopy performed on cycle day 5 versus day 14. To emphasize this concern, Halme et al.,[1] in 1984, published results

that suggested "that female peritoneal macrophages are continuously responding to stimuli."

The authors do not provide clear information about the concentration of peritoneal fluid that was studied. It is known that high concentrations of peritoneal fluid are toxic to gametes and embryos. Thus a major question must be raised: Were the observed differences in the sperm penetration assay secondary to a specific effect on sperm function or were they secondary to a nonspecific toxic effect on sperm, perhaps as demonstrated by altered sperm motility?

Based on these considerations, how did the authors determine the concentration of peritoneal fluid and were observations of sperm motility made?

Since it has been previously demonstrated in endometriosis patients that peritoneal macrophages are morphologically different and exhibit different biologic behavior compared with those in nonendometriosis control patients, research to clarify specific mechanisms is of paramount importance. Based on this study, it is not possible to state unequivocally that peritoneal macrophages interfere with sperm-egg interaction.

It is hoped that the authors will continue to pursue this research in a patient population with better documentation of clinical status, for, when one reviews the literature on peritoneal fluid macrophages, there is a distinct feeling that the answer to one of the most perplexing questions in medicine is near.

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Dr. Chacho (Closing). Several points were made, and I will try to address the most salient ones. We designed this project as a preliminary study to look at several different aspects of peritoneal fluid in patients both with and without endometriosis. In order to obtain a significant number of samples our patients were selected from the practices of five physicians at Michael Reese Hospital and Medical Center. Most of these physicians tended to perform laparoscopy in their patients during the luteal phase of the cycle; therefore there was a considerable difference in the number of samples obtained in the luteal phase compared with the number in the follicular phase. We had no control over the time these physicians brought their patients into the hospital for operation.

Similarly, only a small number of these patients were actually to undergo tubal sterilization. The majority were infertile.

We do have the data as to which patients with endometriosis were infertile and which were fertile. We have not looked at that portion of the data yet.

With respect to several of the other comments concerning some of the problems that are evident in this work, I reemphasize that this was a preliminary study. We are undertaking additional studies at this time with patient groups that are more controlled. We are also looking at the possibility of a problem with cell viability. In the present study we did find that before and after incubation there was no significant difference in the number of viable cells. However, as some of the commenters mentioned, the effects being observed could be those of toxic substances. We are currently looking at cells that have been heat-killed to see if they release into the media anything that might affect sperm penetration.

Prostanoids were assayed in only five patients with endometriosis and in five disease-free women. This was a result of the findings that appeared in the literature during the course of our study. The papers by Sgarlata et al. and Dawood et al. were not in the literature when we began to organize this research. Dr. Sgarlata's paper appeared in 1983, and I first heard Dr Dawood present his work the same year. Since both of these authors confirmed the work of Rock et al. and since our preliminary data also indicated that there was no difference in prostanoid levels between endometriosis patients and women without this disease, we decided not to pursue the prostaglandin analysis. The antisera are extremely expensive, and we did not think it prudent to continue to look at prostaglandin levels when other investigators also had found no elevation in endometriosis patients. We decided to concentrate our efforts on the macrophage studies.

The model discussed in the paper was a benefit of the statistical analysis. The statistician had developed, with the use of discriminate analysis, a model by which we might be able to predict which patients had endometriosis and which patients did not, based on several findings. The particular finding that the model predicted to be most significant was the macrophage concentration. It also predicted that the macrophage content, the concentration of activated macrophages, and the total content of activated macrophages would be significant, and using these particular criteria we made the predictions.

Obviously this appears to be circular reasoning. If you take a group of patients and make a model and then use the model to evaluate that same group of patients you might expect the result to be 100%. However, statistically there is value in such a model. We have not placed too much emphasis on the model at this time since it has not been tested prospectively. There were two patients that the model predicted should have endometriosis, and yet none was seen. To date neither of them has shown any sign of developing endometriosis. We will continue to follow up these patients and are planning to look at other women prospectively. The question was asked as to whether a thorough diagnostic laparoscopy was performed. In most cases I was present in the operating room during the laparoscopy and was able to examine the pelvis. In general the majority of our patients were infertile and consequently a thoroughly diagnostic laparoscopy was performed by the primary physician. We did make a special point of

doing a thorough diagnostic laparoscopy in those patients who were having tubal sterilization. We did not accept any patients who were using steroids or intrauterine contraceptive devices for contraception. All of the patients were using various barrier methods of contraception before the tubal ligation.

A question was asked about the concentration of peritoneal fluid. From this I assume the question concerned the concentration of peritoneal fluid used in our various assays. We obtained 3 ml of peritoneal fluid, isolated the macrophages with the use of ficoll-diatrizoate, and then determined the concentration of macrophages in that particular fluid. We brought those macrophages to a concentration of 1.5×10^6 cells per milliliter in fresh medium. We did not put them back in the peritoneal fluid. Therefore, the peritoneal fluid itself, unless it was closely adherent to the macrophages, should not have been present in our incubates nor should it have been able to affect the penetration as measured by our sperm penetration assay.

Conservative treatment of ectopic pregnancy with methotrexate

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Chicago, Illinois

Six subjects with distal ampullary ectopic pregnancies were treated with four doses of intravenous methotrexate (1.0 mg/kg) followed by four doses of leucovorin (0.1 mg/kg, intramuscularly). The diagnosis was established in all cases by laparoscopy following sonography and radioimmunoassay for serum β subunit of human chorionic gonadotropin. Subjects were followed with daily quantitative serum β-human chorionic gonadotropin radioimmunoassay and sonography. Five of the six subjects experienced resolution of their ectopic pregnancy without additional surgical treatment. One subject underwent salpingectomy following treatment. Morbidity also included three patients with mild stomatitis or gastritis, and two patients had transient elevations of serum transaminase levels. Two patients had protracted courses and received blood transfusions. The most abrupt response and most uncomplicated courses were experienced in the three subjects with initial human chorionic gonadotropin levels below 1000 mIU/ml. This preliminary experience suggests that methotrexate may be an effective alternative for the treatment of early ectopic pregnancy. (Am J Obstet Gynecol 1986;154:1299-1306.)

Key words: Ectopic pregnancy, methotrexate, infertility

The incidence of ectopic pregnancy in the United States increased threefold during the 1970s with over 52,000 cases reported during 1980.[1] During this same interval the death-to-case ratio declined from 3.5 to 0.9; only 39 deaths occurred in the United States in 1979. The reasons for this dramatic increase in incidence have not been fully elucidated, but the prevalence of pelvic inflammatory disease and the possible contribution of intrauterine device use have been cited as contributing factors. The availability and liberal use of sensitive and specific radioimmunoassays for β subunit of human chorionic gonadotropin (β-hCG), high-resolution sonography, and laparoscopy have permitted earlier and more frequent diagnosis and have resulted in a significantly reduced mortality rate.

Despite this apparent success in diagnosis and treatment, fertility remains impaired. Term pregnancy following ectopic pregnancy occurs in only 30% of patients traditionally treated with salpingectomy.[2-4] This observation has led to the development of a variety of surgical techniques including linear salpingotomy, segmental resection and reanastomosis, and tubal abortion to conserve the oviduct and future fertility potential. Those procedures utilizing microsurgical techniques have resulted in slightly improved fertility, with investigators reporting success rates ranging from 40% to 72%.[5-7]

These observations make conservative nonsurgical

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Table I. Patient characteristics

Subject No.	*Age (yr)*	*Weight (kg)*	*Height (cm)*	*Last menstrual period to presentation (wk)*	*Gravidity*	*Parity*	*Previous ectopic pregnancy*	*Previous therapy*	*Initial hCG level (mIU/ml)*	*Laparoscopic findings*
1	33	50	157	6⅐	3	0	1	Right linear salpingotomy	648	Right ampullary ectopic, 3 × 2 cm
2	27	98	179	6	5	2	1	Left salpingectomy	103	Right ampullary ectopic, 3 × 2 cm
3	28	80	163	15	5	1	0		187	Left ampullary ectopic, 3 × 2 cm
4	28	68	163	7⅐	3	0	1	Right linear salpingotomy	25,410	Right ampullary ectopic, 3 × 3 cm
5	23	45	160	5⅐	3	1	1	Left salpingectomy	8,025	Right ampullary ectopic, 2.5 × 1 cm
6	32	52	160	6⅐	2	1	1	Left salpingectomy	16,170	Right ampullary ectopic, 2.5 × 2 cm

therapy appealing. Such an approach would spare patients laparotomy and a conservative tubal operation and might result in less tissue trauma and subsequent adhesion formation if the ectopic pregnancy could be induced to resolve spontaneously. Ultimately, reproductive function might be less adversely affected.

Recently a case report appeared describing the successful use of methotrexate in the treatment of an interstitial pregnancy.[8] This approach was applied in the present study to determine the effectiveness and safety of methotrexate therapy in the treatment of tubal ectopic pregnancy in a series of patients.

Material and methods

Between September 1, 1984, and August 31, 1985, 52 patients presenting at Northwestern Memorial Hospital with suspected tubal ectopic pregnancies were given the option of participation in a protocol using methotrexate for treatment of ectopic pregnancy. In the sample the diagnosis of tubal ectopic pregnancy was subsequently established by β-hCG radioimmunoassay, ultrasonography, and laparoscopy. The study was explained and written informed consent was obtained before laparoscopy. An Investigational New Drug License was secured from the United States Food and Drug Administration to use methotrexate to treat tubal ectopic pregnancy before inception. The treatment protocol and consent forms were reviewed and approved by the Northwestern University and the Northwestern Memorial Hospital Institutional Review Boards and the Prentice Pavilion Research Committee.

The final decision regarding inclusion in the study was made at the time of laparoscopy. Inclusion criteria included the presence of an unruptured tubal ectopic pregnancy ≤4 cm in diameter and with <100 ml of blood in the peritoneal cavity. Photographs of the ectopic gestations were taken at laparoscopy. Subjects were excluded if they had unstable vital signs, a contraindication to laparoscopy or indications for additional abdominal surgery, a history of active hepatic or renal disease, abnormal serum blood urea nitrogen, creatinine, or transaminase levels, proteinuria, methotrexate sensitivity, active peptic ulcer disease, a white blood count of 3000, a platelet count of <10,000, or a hemoglobin of <11.0 grams of if they were deemed to be poor candidates for follow-up.

Of the original 52 patients, six subjects agreed to the protocol, signed the consent form, and satisfied the inclusion criteria. All six subjects were transferred to the Northwestern Memorial Hospital Clinical Research Center immediately after laparoscopy. All individuals were between 18 and 35 years of age, and all desired future pregnancies. The first dose of methotrexate was administered when vital signs were stable. A total of four doses of methotrexate (1 mg/kg) was given every other day. Leucovorin (citrovorum factor, 0.1 mg/kg) was given intramuscularly every other day following methotrexate administration for a total of 4 days to minimize toxicity.

Sequential Multichannel Autoanalyzer (SMA-20) determinations were repeated before each dose of methotrexate. Quantitative β-hCG radioimmunoassay, complete blood count determinations, and ultrasound studies were performed daily to evaluate response to therapy. Serum methotrexate determinations were obtained 2, 4, 24, and 36 hours after the initial dose of methotrexate. Twenty-four–hour urine collections for creatinine clearance and protein were performed the

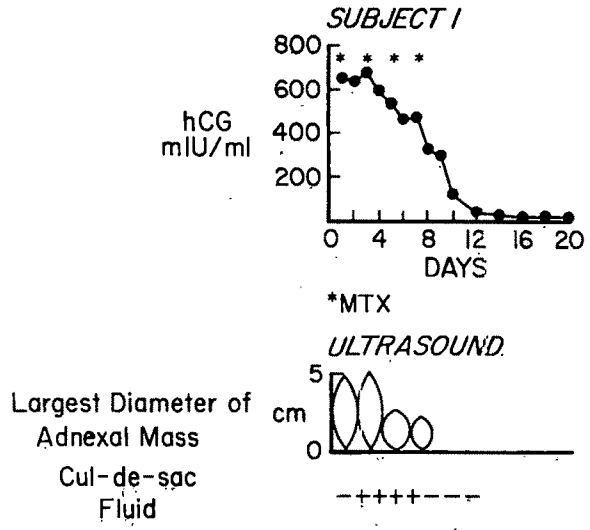


Fig. 1. Human chorionic gonadotropin *(hCG)* levels and ultrasound findings for subject 1.

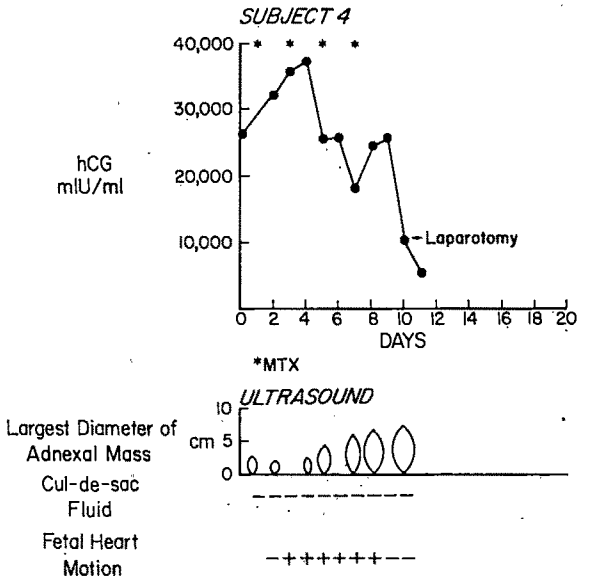


Fig. 2. Human chorionic gonadotropin levels and ultrasound findings for subject 4, the treatment-failure patient.

Table II. Adverse effects

Effect	*Subject No.*
Stomatitis	1, 3
Gastritis	5
Abnormal serum glutamic-oxaloacetic transaminase	2, 3
Abnormal lactic dehydrogenase levels	2
Protracted course	5, 6
Transfusion	4, 5, 6
Treatment failure	4

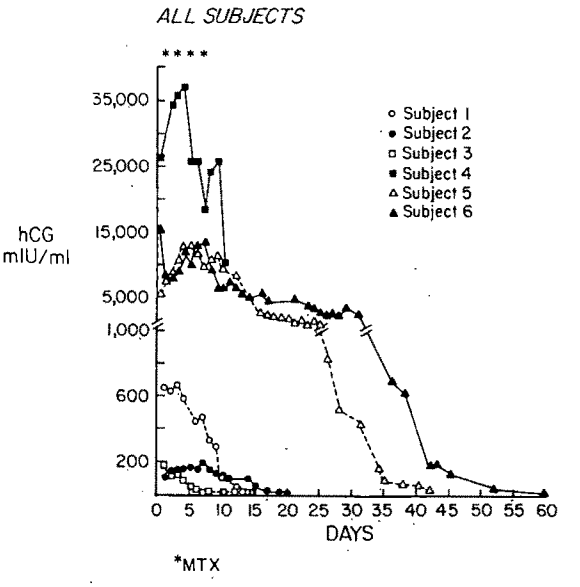


Fig. 3. Composite pattern of all subjects' levels.

first full day of admission and three months after therapy. A hysterosalpingogram was performed 3 months following therapy to assess tubal patency.

Patients were discharged from the Clinical Research Center when objective evidence of tubal abortion or resolution was obtained with falling hCG levels and sonographic date. They were advised to avoid subsequent pregnancy for 3 months following treatment.

Results

The patient characteristics are presented in Table I. Interestingly, five of the six subjects had a previous ectopic pregnancy. Representative hCG patterns and sonogram results are presented in Figs. 1 and 2. Fig. 3 shows the composite hCG levels of all six subjects. Five of six patients had complete resolution of their tubal pregnancies with methotrexate alone. Subjects 1, 2, and 3 had relatively rapid responses to methotrexate therapy and no significant complications (Table II). The single failure was subject 4, who developed a hemoperitoneum and acute abdomen requiring operation on therapy day 12. At operation the fallopian tube was noted to be intact and the tubal gestation was free in the peritoneal cavity; however, arterial bleeding from the tubal placental implantation site was present. There were approximately 400 ml of blood in the abdomen. The patient underwent a partial salpingectomy and had an uneventful postoperative course.

Subjects 5 and 6 had resolution of their ectopic pregnancies without laparotomy but also had complicated courses. Subject 5 had moderate abdominal pain accompanied by vaginal bleeding but remained hemodynamically stable. This episode appeared to represent a tubal abortion (judged by falling β-hCG levels, sonography, and clinical examination). One day following this episode, the patient improved dramatically, but a

2.5 gm decrease in hemoglobin since admission was still noted. She therefore received two units of packed red blood cells and plans were made to take her to the operating room. However, no further decrease in her hemoglobin and hematocrit was noted. She continued to improve clinically, and additional surgery was not performed. Subject 6 had a similar episode 24 days after beginning treatment. This prompted readmission and transfusion. She experienced rapid clinical improvement following these measures.

Other complications were minimal. Subjects 1 and 3 complained of mild stomatitis, which spontaneously resolved within 3 days of appearance. Subjects 2 and 3 had transient elevations of serum glutamic-oxaloacetic transaminase, and subject 2 also had an elevation of serum glutamic-pyruvic transaminase levels during therapy. Abrupt resolution was noted in both cases while treatment was continued. No other morbidity was observed.

Comment

Our preliminary data suggest that methotrexate may be effective in inducing tubal abortion or resorption of ectopic pregnancy; five of six subjects avoided operation. The agent appears to be most effective in pregnancies with β-hCG levels of <1,000 mIU/ml. The subjects experiencing the most complicated courses (4, 5, and 6) had significantly higher initial β-hCG levels and presumably more advanced gestations.

Conservative management was attempted in a large series of patients in Denmark in 1955.[9] In this series 68 of 119 patients (57%) with the presumptive diagnosis of ectopic pregnancy were managed without surgical intervention. In a more recent series, four of five patients with early ectopic pregnancy were successfully managed without surgery.[10] The primary problem with observant management in the Danish study was the need for prolonged hospitalization; 60% of the patients were hospitalized for over 30 days. Tanaka et al.[8] more recently described successful resolution of an interstitial ectopic pregnancy following methotrexate therapy.[8] This approach resulted in a more abrupt termination of the pregnancy, and tubal patency was preserved, as demonstrated by subsequent hysterosalpingography.

If methotrexate is used, daily quantitative β-hCG radioimmunoassay determinations appear to be a reliable parameter for following the resolution of an ectopic pregnancy. Ultrasound was also helpful when performed daily; however, ultrasound lacked the sensitivity of the β-hCG radioimmunoassay levels. It was most helpful in predicting the lack of response of subject 4. The size of the oviduct was not helpful in predicting hCG levels, since all patients had tubal pregnancies of <4 cm in diameter. The single treatment failure, subject 4, had an initial β-hCG level of 25,410 mIU/ml, and fetal heart motion was noted within the oviduct on treatment day 3. In retrospect, the subject's course might have been predicted by these findings and the delay in the decline of hCG levels (Fig. 2). Most subjects had an episode that clinically appeared to represent the tubal abortion or resorption. This event consisted of moderate abdominal pain, vaginal bleeding (in some instances with the passage of a decidual cast), and a near-linear fall in β-hCG levels following the subjective signs.

Although our results do not permit us to predict a typical hCG regression curve for resolution of a tubal pregnancy, the fall in the hCG levels of subject 3 are probably coincidental with, rather than a result of, methotrexate therapy. This subject was probably undergoing a spontaneous tubal abortion at the time of diagnosis.

What is the role of methotrexate? If a proportion of the current "epidemic" of ectopic pregnancies reflect ectopic pregnancies that in the past would have spontaneously resolved early or without sequelae or clinical detection, there may be some merit in attempting to avoid surgical intervention in these situations. Observation alone may be justifiable if hCG levels are decreasing and if the patient is clinically stable.[10] It is unlikely that methotrexate therapy will become the most common treatment for ectopic pregnancy. Our preliminary experience suggests that the agent can provide a safe and relatively effective alternative to surgery for patients with early ectopic pregnancies. Methotrexate therapy may ultimately prove to be a cost-effective alternative to surgery. We do not recommend the use of methotrexate when an ectopic pregnancy is associated with hCG levels of >10,000 mIU/ml or when fetal heart motion can be detected. Although it may be effective in more advanced tubal pregnancies, the more protracted course that we observed in those subjects makes it considerably less appealing. Considerable additional experience must be accumulated to determine safe hCG limits within which to employ therapy and to assess subsequent fertility following methotrexate treatment. If fertility in patients treated with methotrexate is significantly improved over that of surgically treated patients, its greatest potential advantage will be realized.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. G. William Bates, Jackson, Mississippi. In my opinion, when a definitive medical treatment replaces a surgical procedure, medical practice has advanced. In this paper, the authors describe the treatment of six women with early tubal ectopic pregnancies with intravenous methotrexate. Five of the six women had a complete resolution of their ectopic pregnancy as measured by the disappearance of hCG from plasma and the radiologic appearance of the fallopian tube 3 months after methotrexate therapy.

These investigators are not the first to report the successful use of methotrexate for the treatment of ectopic pregnancy. Tanaka et al.[1] described the successful treatment of an interstitial ectopic pregnancy with subsequent demonstration of a patent fallopian tube in 1982; Farabow et al.[2] reported the successful treatment of a woman with a cervical pregnancy using methotrexate in 1983. In Farabow's case, surgical treatment would have mandated a hysterectomy and consequent loss of reproductive function. We reported the successful treatment of a woman with a tubal ectopic pregnancy 3 months after a tubal anastomosis.[3] In our case, hCG disappeared from serum 15 days after the initiation of methotrexate.

Ory and associates are the first investigators to develop a protocol for selecting and managing women with early ectopic tubal pregnancies, and they are the first to report a series of women treated with methotrexate. Five of the six women in their series (83%) had complete resolution of their ectopic pregnancy.

Methotrexate has been used for the treatment of gestational trophoblastic disease for nearly 30 years. Li et al.[4] first reported the use of methotrexate for the treatment of choriocarcinoma, choriadenoma, and hydatidiform mole in 1956. Since that report, methotrexate has been the standard treatment for gestational trophoblastic disease. Why has it taken so long to apply this successful medical therapy to the problem of ectopic pregnancy? Ory and associates answer this question in their paper. Advances in the technology of sensitive and specific radioimmunoassay for hCG and improved resolution of ultrasonography have recently enabled the obstetrician to suspect an ectopic pregnancy before the pregnancy ruptures from its implantation site. The diagnosis of suspected unruptured ectopic pregnancy can be confirmed by diagnostic laparoscopy. Bryson[5] made a prospective study of 81 women by means of hCG assay and ultrasonography; he reported an accuracy of 100% in selecting women with an early, unruptured ectopic pregnancy.

Before the application of these diagnostic tools, ectopic pregnancy was only suspected when a woman presented with signs of hemoperitoneum from a ruptured fallopian tube. In these women, surgical resection of the ruptured fallopian tube was the only available treatment.

With the improvement in the clinician's ability to make the diagnosis of an early unruptured ectopic pregnancy, salpingectomy has given way to linear salpingotomy or "milking" the pregnancy from the tube.[6] The long-term success rate of this treatment, that is, repeated ectopic pregnancy or improvement in fertility is still uncertain. However, Rivlin et al.[7] and Richards[8] reported persistent trophoblastic tissue following conservative surgical management, which subsequently necessitated salpingectomy for their patients. Perhaps methotrexate treatment would have spared the fallopian tubes of these women.

It is important to point out the rising incidence of ectopic pregnancy in the United States. Rubin et al.[9] reported that the incidence of ectopic pregnancy rose from 4.5 per 1000 reported pregnancies in 1970 to 9.4 per 1000 reported pregnancies in 1978. The risk of an ectopic pregnancy is highest in nonwhite women and women aged 35 to 44 years. The rising incidence of ectopic pregnancy has been attributed largely to the rising incidence of gonococcal and chlamydial salpingitis.

I would like to ask the authors two questions. First, in Table I of the article, all subjects were listed as having had an ectopic pregnancy. Was this the ectopic pregnancy managed with methotrexate by the authors, or had these subjects had a prior ectopic pregnancy? If the ectopic pregnancy treated with methotrexate was the second ectopic pregnancy for each subject, did this fact influence the patients and the authors to select medical rather than surgical theapy? Would the authors select methotrexate therapy for a first ectopic pregnancy?

Second, the authors obtained a hysterosalpingogram on each subject 3 months after the completion of medical therapy. What was the result of the posttreatment hysterosalpingogram?

In my opinion this study is an important contribution to our methods for management of ectopic pregnancy. With improved diagnostic tools to select women with ectopic pregnancies before rupture, it is reasonable to think that methotrexate therapy could become the primary method of treatment for this pregnancy complication. Moreover, this study should encourage obste-

tricians to consider more carefully the risk factors for ectopic pregnancy and to perform early ultrasonography in women at risk for ectopic pregnancy. Finally, with the case reports of persistent trophoblastic tissue in women undergoing conservative surgery for ectopic pregnancy, we should obtain serum hCG titers following conservative surgery to select women with persistent trophoblastic disease and treat them with methotrexate. Holtz[10] has constructed disappearance curves of hCG in women following linear salpingotomy.

The authors should continue these studies to enlarge their series and evaluate the long-term consequences of methotrexate therapy. Other investigators should make independent evaluations of methotrexate therapy for unruptured ectopic pregnancy.

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DR. LOUIS WEINSTEIN, Tucson, Arizona. Since many obstetric and gynecologic therapies are controversial, it is a pleasure to be able to discuss a manuscript in which there is no controversy regarding the proposed therapy. Simply stated, the use of a potent antineoplastic, antimetabolite drug, methotrexate, for treatment of an ectopic pregnancy is inappropriate and potentially dangerous.

The authors have presented a report of six patients with the laparoscopic diagnosis of an ectopic pregnancy who received a four-dose course of intravenous methotrexate for therapy. The facts of the six cases are as follows. One patient developed an acute abdomen requiring operation and hemoperitoneum on day 12 of therapy, requiring an exploratory laparotomy. Two patients had abdominal pain and a significant hemoglobin drop after their course of methotrexate therapy and required blood transfusions. One of these episodes occurred 4 weeks after the start of therapy. All of the remaining patients experienced stomatitis and/or elevated liver enzyme levels. Of the three patients stated to have a successful course of therapy, one appeared to be experiencing a tubal abortion at the time the treatment was started. These data therefore reveal a success rate of 33% and a morbidity rate of 100%.

It is difficult to determine from the report the average length of hospital stay, but it was at least 8 days or longer, since the four-dose course of methotrexate was given every other day. Are the authors advocating an 8- to 10-day hospital course for an ectopic pregnancy or do they want the clinician to treat the patient with an ectopic pregnancy as an outpatient, returning to the office every other day for intravenous methotrexate therapy?

Equal to my concern for the inappropriateness of the methotrexate therapy is the ethics of performing this study. Before the start of the methotrexate therapy each patient underwent a laparoscopy to confirm the diagnosis of the ectopic pregnancy. I am baffled in my attempt to visualize how an institutional review board could approve such a project, and how adequate informed consent could be given to these patients. I do not understand what would motivate a patient to agree to a hospital stay of more than 8 to 10 days, a general surgical procedure, daily blood drawing, and the administration of a potent antineoplastic agent when a simple surgical procedure could cure her problem.

The last paragraph of the article states that although "it is unlikely that methotrexate therapy will become the most common treatment for ectopic pregnancy . . . our preliminary experience suggests that the agent can provide a safe and relatively effective alternative to surgery. . . ." I wish to remind the authors of a statement from the nineteenth century scientist, Jules Henri Poincaré, who said, "Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house."

I should appreciate it if the authors would address the following points: (1) What was the length of hospital stay for the five patients? (2) What was the cost of total therapy for each patient and who was responsible for the bill? (3) Was this study reviewed by their institutional review board and was any difficulty encountered in getting approval? (4) How and by whom was the study presented to the patient? (5) How long was the consent form and was any financial renumeration offered to the patient for her participation?

DR. HARRY K. WADDINGTON, Olympia Fields, Illinois. In addition to the suggestion of taking salpingograms, in spite of the penetrative process of laparoscopy, I think we should have been shown photographs of the interior of these abdomens taken by laparoscopy of the four patients who did not have laparotomy. Otherwise, this is an excellent presentation.

DR. ROBERT A. MUNSICK, Indianapolis, Indiana. I think Dr. Weinstein is right, that it is unusual and

strange that this was apparently approved by an institutional review board; but the fact is, it was. Therefore I wonder why there was no control group to determine whether they also had an approximately 50% success rate, which I believe they probably would have had with diagnoses made this early.

I also would like to remind the authors that about one in 300 ectopic pregnancies will have a concomitant combined intrauterine pregnancy, and I would hate to give methotrexate to that patient.

Dr. Eduard Eichner, Cleveland, Ohio. First of all, I disagree with Dr. Weinstein in this regard. It was a tough situation, but if you use private patients, I have found in my research over many years that a private patient will do almost anything the physician wants her to do and will sign all the papers necessary; if you can explain to the pateint that this is a method that might save other patients' lives and major operations, they will go along with it willingly.

I am interested in two other things: I took my early training at a time when we were not permitted to operate on an unruptured ectopic pregnancy because that was considered to be an abortion. Now, with the right-to-life movement getting very strong, the destruction of a fetus in the abdomen by destruction of the chorionic tissue and so on, will bring them to the fore. Will the hospital be able to handle it?

The final question I want to ask concerns reconstruction of the tube or the viability of the tube. Did any of these patients have a successful intrauterine pregnancy after the procedure?

Dr. Madhwa Raj, New Orleans, Louisiana. This is a very interesting presentation. It occurred to me that perhaps the best course would be to look for suitable animal models that we could use experimentally without all these problems. As you know, there have been several models in the works for ectopic production, including one in the primate, so I would put before this audience the suggestion that we seriously consider doing some more basic work on primates and making sure that these hurdles can be safely crossed.

Dr. Ory (Closing). I appreciate the spirited comments.

Regarding Dr. Bates' initial questions regarding whether or not a previous ectopic pregnancy changed our management, as I pointed out, five of the six did have previous ectopic pregnancies; two of them were managed conservatively and three with salpingectomies. Patients who had had conservative procedures and had recurrent ectopic pregnancies were certainly more amenable to trying a different approach. We presented the option that if the patient had a recurrence in an affected oviduct, it might be more desirable for them to have the tube removed, and some of the 52 excluded patients were omitted on that basis.

Regarding the hysterosalpingograms, I had hoped to have six of them completed and available to show you. Many of these subjects came into the study rather late and are just reaching their 3-month point now. In the two that we have performed, one in the first group had preservation of tubal patency. The second one, who was subject 5, did have distal tube occlusion. There was quite a bit of adhesive disease noted at the time of initial laparoscopy, however.

Regarding Dr. Weinstein's comments, the length of hospitalization ranged from 8 to 25 days. One subject, No. 5, was readmitted after discharge when additional concerns regarding a gradually falling hemoglobin level developed.

This was a pilot study. It was funded by a National Institutes of Health Clinical Research Center grant, in part. In order to undertake this, we felt it was critical to monitor these patients as intensively as possible, which we did. We are not proposing that this be done on an outpatient basis. As I mentioned, I think it will be best suited for patients with very early ectopic pregnancies. I do not think we have enough experience, as yet, to say whether an outpatient modality will be viable, but it may well be an option.

As I mentioned, the cost was borne in part by the Clinical Research Center and in part by the patients themselves. The total cost including laparoscopy, hospitalization, testing, and so on, ranged from about $6,200 to $8,500, which is consistent with the comprehensive cost of salpingectomy or linear salpingotomy in our institution.

The protocol was formulated after 18 months of work. The FDA was involved in developing the protocol, and we were granted the first investigational new drug license in the United States to test the use of methotrexate for this purpose. They had several suggestions regarding monitoring toxicity, but they were enthusiastic about this application. Lederle, the manufacturer of methotrexate, was also involved in obtaining the license and was quite helpful as well.

Locally, the protocol was approved by separate hospital and university institutional review board committees, and there was extensive modification over a 6-month period of time. There was no outright objection to implementing the protocol, only suggestions for additional refinement.

I personally presented the protocol and consent form to each patient. There was no remuneration offered whatsoever. Photographs were taken of all patients at the time of laparoscopy.

The issue of the control group is an interestng one, and one that we discussed at length. There is a strong clinical impression that the incidence of spontaneous tubal abortion is more frequent than we suspect. No one, at least when we initiated this study 18 months ago, was prepared to simply observe patients with ectopic pregnancies without treatment. Our institutional review board did object and were also unwilling to subject patients to that. Consideration is being given to observant management, and if patients with suspected ectopic pregnancies are followed for a few days with decreasing quantitative hCG levels, it may be reasonable to delay surgery and see whether these patients will not undergo spontaneous resolution. In fact, we have had the opportunity to observe this phenomenon

in four or five patients at Northwestern, and there are clinical protocols in progress to test this on a broader scale.

With regard to the risk of inadvertently administering methotrexate to a woman with concomitant intrauterine pregnancy, that possibility was excluded by ultrasound studies. We did not routinely submit these patients to curettage because we were interested in seeing whether they did pass decidual casts and whether that might be clinically useful in monitoring their response.

We have not had any subsequent term pregnancies in this group; none of these women subsequently conceived. Each was instructed to use contraception for 3 months after the study. This was to enable the methotrexate to be cleared and to allow the oviduct to completely heal.

Finally, regarding Dr. Raj's comment about the animal model, I understand the rabbit is a reasonable model to work with in ectopic pregnancies. However, I think the clinical parameters we were evaluating here could not have been satisfactorily tested.

Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings

Frank O. Page, M.D., James N. Martin, M.D., Sue M. Palmer, M.D., Rick W. Martin, M.D., John A. Lucas, M.D., G. Rodney Meeks, M.D., Edsel T. Bucovaz, Ph.D., and John C. Morrison, M.D.

Jackson, Mississippi

In this study the immediate neonatal acid-base status, obtained via a double-clamped segment of umbilical cord, in 75 term, singleton vaginal deliveries was compared to electronic fetal heart rate recordings and Apgar scores. Of 75 neonates, 59 had 1-minute Apgar scores ≥7 and 52 had an initial pH >7.20. Six of the 16 neonates with a 1-minute Apgar score <7 demonstrated a low pH (<7.20). At 5 minutes only eight of 75 neonates had Apgar scores <7 with six of the eight having pH values <7.20. Of those neonates with Apgar scores ≥7 and pH <7.20 (seven neonates at 1 minute, two at 5 minutes), none had metabolic acidosis. Eighteen fetal heart rate tracings were considered abnormal; acidosis was confirmed in eight (44%) by pH criteria, yet only three of the eight neonates had low Apgar scores. Our investigations suggest that the combination of fetal heart rate monitoring, cord blood pH, and Apgar assessment is better than any one parameter alone as an evaluation of fetal status just after delivery. (Am J Obstet Gynecol 1986;154:1306-11.)

Key words: Neonate, acid-base status, Apgar scores, fetal heart rate, correlation

Before the development of Apgar scoring, there was no systematic method for neonatal assessment immediately after birth. The original intentions of this scoring system were to predict immediate neonatal survival, to evaluate various methods of resuscitation, to compare the early perinatal experience in different hospitals, and to ensure closer observation of the infant during the first few minutes of life.[1] In the 30 years since its introduction, the Apgar score has become one of the most important evaluation systems in medicine for the early identification of neonatal complications.[2]

Unfortunately during the past two decades neonatal Apgar scores at 1 and 5 minutes have been considered by some to reflect intrauterine asphyxia and to predict long-term neurological outcome. The Apgar score, however, is often poorly correlated with other indicators of intrauterine well-being.[3] To better and more accurately reflect intrauterine fetal status and distress, many other evaluation methods have been studied, including cord blood pH, lactate, arginine vasopressin, isoenzymes, and catecholamines as well as fetal scalp blood sampling, fetal scalp stimulation, passage of meconium, and electronic fetal heart rate assessment. Of these methods, electronic fetal heart rate assessment is a good predictor of fetal health but is associated with a high false positive rate when forecasting asphyxia and

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Table I. Apgar scores versus pH values

Apgar score	*pH*	*1 min*	*5 min*
≥7	≥7.20	52*	65
	<7.20	7	2
<7	≥7.20	10	2
	<7.20	6	6

*p < 0.02 at 1 minute.

Table II. Abnormal fetal heart rate tracings (n = 18)

		Apgar score ≤7	
pH	*n*	*1 min*	*5 min*
≥7.20	10	1	0
<7.20	8	3	1
>7.20, <7.30	15	1	0

is even more deficient in predicting long-term outcome.[4,5] Neonatal arterial blood gas and pH assessment in the nursery correctly reflect acidosis but usually do not correlate well with Apgar scores or electronic fetal heart rate findings, perhaps in part because they are usually obtained remote in time from the delivery process.[6] Umbilical cord blood gas values at delivery have been correlated with Apgar scores, are easier to obtain than fetal scalp samples, and are more timely than later neonatal studies.[7] The purpose of this study was to investigate the correlation of 1- and 5-minute Apgar scores with umbilical arterial blood gas values and electronic fetal heart rate recordings.

Material and methods

This prospective study was performed during a 3-month period from July 1, 1984, to September 30, 1984, in the perinatal unit of the University of Mississippi Medical Center. The umbilical arterial acid-base status, Apgar scores, and intrapartum electronic fetal heart rate tracings were obtained from 75 randomly selected, singleton, term, vaginal deliveries.

An 8-inch segment of umbilical cord was doubly clamped immediately after delivery of the infant. Blood from the umbilical artery was withdrawn into a 1 ml plastic syringe that had been preflushed with a heparin solution (1000 units/ml). Each blood-filled syringe was placed in ice for transport to the laboratory. Arterial blood gases and pH determinations were performed within 20 minutes of delivery with an IL-813 blood gas analyzer. The standard definition of neonatal acidosis with a pH value of <7.20 was used.[8] Apgar scores were determined 1 and 5 minutes after birth by trained delivery room nurses (who assign scores to all infants). An Apgar score ≥7 was considered normal.

All patients were monitored electronically by means of a fetal scalp electrode during the intrapartum period. The electronic fetal heart rate tracings were interpreted in a blinded fashion by an observer without knowledge of the intrapartum course, blood gas data, neonatal outcome, or Apgar scores. Any of the following alterations in the fetal heart rate tracing were considered abnormal: late decelerations after 50% of contractions during 30 minutes, significant variable decelerations (<60 bpm for 60 seconds), minimal beat-to-beat variability for 30 minutes (<3 bpm), and/or prolonged bradycardia (<100 bpm for >2 minutes).

The electronic fetal heart rate recordings and newborn hospital records were examined in order to determine any correlation with Apgar scores and neonatal acid-base values.

The χ^2 analysis was used to determine statistical significance at a level of <0.05.

Results

Study subjects ranged in age from 14 to 39 years with a mean of 23 years. The average gestational age was 39 weeks with a range of 37 to 42 weeks. Forty-eight of the infants were delivered spontaneously while 27 were delivered with elective outlet forceps. There were 39 male infants included in the study and 36 female infants. One third of the patients were delivered while under regional anesthesia while others received pudendal, local, or no anesthetics. None of these factors were significantly different when normal versus abnormal Apgar scores, cord pH values, or electronic fetal heart rate tracings were compared. Review of the neonatal hospital course infant charts revealed no significant morbidity, except for one infant with a major congenital anomaly that required a corrective surgical procedure. Four infants had transient hyperbilirubinemia that required phototherapy.

Fifty-nine of the 75 neonates (79%) had 1-minute Apgar scores of ≥7 (Table I). Of the 59 neonates, seven (12%) had an umbilical arterial pH of <7.20, while 52 (88%) had an initial pH of ≥7.20. When the Apgar score was ≥7 there was a significantly greater frequency of pH values >7.20 than expected by chance alone (p = 0.02). Sixteen of the 75 neonates (21%) had a 1-minute Apgar score of <7. Ten of the 16 (62%) with low Apgar scores had an initial pH of ≥7.20 and six (38%) had an initial pH of <7.20. When the 5-minute Apgar scores were studied, only eight of 75 infants had scores ≤7. Of these eight infants, six had pH values <7.20, while only two of 67 infants (2.9%) had 5-minute Apgar scores ≥7 and were acidotic by pH criteria. Review of the acid-base status in both of these neonates revealed an acute respiratory acidosis (a low pH with a high P_{CO_2} and a normal base deficit).

There were 18 electronic fetal heart rate recordings

that were considered abnormal by the previously stated criteria. Acidosis was confirmed by pH criteria in eight of these 18 infants (44%) (Table II). If the 1-minute Apgar score was considered, only three of the eight acidotic infants would have been identified if one assumes Apgar scoring to reflect acidosis. Of 18 abnormal tracings, 15 (83%) were associated with pH values >7.20 but <7.30.

Comment

The value of the Apgar score as a systematic method to generally assess newborn well-being is accepted as an extremely important tool.[9] Because the Apgar scoring system does not often reflect the degree of acidosis at delivery, its value as an indicator of perinatal asphyxia has been questioned.[3, 5, 10] Sykes et al.[3] summarized the findings of these relationships in more than 200 consecutive deliveries. In that study, 21% of the neonates with a 1-minute Apgar score of <7 had severe acidosis at delivery as evidenced by an umbilical arterial pH of <7.10 and a severe base deficit. On the other hand, 75% of the infants with a significant acidosis had an Apgar score ≥7 at 1 minute. In our study 38% had acidosis if the Apgar score was <7 at 1 minute.

Hon and Khazin[11] have shown that fetal scalp pH correlates with Apgar scores but that there is considerable overlap between pH values for infants with high and low Apgar scores. Their work suggests that if pH <7.20 is used to delineate acidosis, 15% to 20% of neonates have a pH >7.20 yet have Apgar scores of 1 to 6 at 1 minute. Fields et al.[12] reported a comparative study of umbilical cord pH values and Apgar scores in 172 singleton neonates. When the Apgar score was <7, 56% of the neonates had a normal pH value, which is very close to the 62% in our own study.

Similarly, umbilical cord pH has been correlated with electronic fetal heart rate and outcome at delivery.[4] Unfortunately, even in the face of high risk pregnancies, Low et al. found that fetal metabolic acidosis was present in only 48% of those with abnormal fetal tracings, similar to the 44% in the current study. The severity of fetal hypoxia as manifested by a significant metabolic acidosis is also a poor predictor of neurological outcome. Low et al.[13] found that only 56% of fetuses with severe fetal hypoxia had newborn encephalopathy. Therefore, acid-base studies alone do not appear to be excellent predictors of long-term outcome nor are they well correlated with newborn condition at birth or with electronic fetal heart rate tracings.

Neurological follow-up of infants with low Apgar scores has likewise been unrewarding. Nelson and Ellenberg[14] studied the correlation of Apgar scores and cerebral palsy in approximately 40,000 infants who were followed up for 7 years. Low Apgar scores were risk factors for cerebral palsy, but 55% of children with later cerebral palsy had Apgar scores of 7 to 10 at 1 minute and 73% had scores of 7 to 10 at 5 minutes. Of the children who had Apgar scores of 0 to 3 at 10 minutes or later and survived, 80% were free of major handicap at early school age.

Our study indicates that intrapartum fetal monitoring patterns and Apgar scores, which are used retrospectively by some to predict biochemical asphyxia in the neonate, have a high incidence of false positive results. Neonates with Apgar scores <7 or abnormal electronic fetal heart rate can be assessed by use of an umbilical arterial analysis to ascertain the presence of acidosis and to differentiate between metabolic and respiratory acidosis. This adjunctive information, while not specific in and of itself for fetal distress, can be used in conjunction with the Apgar score and electronic fetal heart rate to give a better assessment of neonatal condition than that with any one modality alone. We recommend the use of umbilical cord acid-base assessment when ominous electronic fetal heart rate tracings are present, unsuspected neonatal distress is noted at delivery (Apgar score < 7), a birth weight <1500 gm is suspected, or other signs of intrapartum distress have been noted (meconium). Although most of the infants in these categories develop normally after birth, these are the conditions that relate to abnormal neurological outcome and litigious action.

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Discussion

Dr. Marvin L. Hage, Durham, North Carolina. Assessment of the health of the newborn infant and prediction of future morbidity and mortality is a critical function of perinatal research and practice. The combined use of electronic fetal monitoring, Apgar scores, and umbilical arterial pH has been described in this paper as well as by others.[1-6] Findings of these studies have shown overall agreement among the three tests; however, the specificity of the tests as to long-term outcome (mortality or morbidity) has been low.[7-9] The controversial question remains as to the addition of umbilical arterial blood gas biochemical testing. Specifically, I would like to ask the authors to comment on the following:

1. Would it be better to measure base excess since that has been correlated with neonatal encephalopathy?[10]
2. Would they recommend simultaneous measurements of maternal pH?[11]
3. Were there any technical problems in performing the test, particularly in obtaining the sample, clotting, or costs of the tests?
4. For institutions that do not have easy accessibility to 24-hour blood gas evaluation, would they recommend any other techniques?
5. What would the authors recommend for premature births, a high-risk area for both neonatal death and morbidity?[12]
6. Do the authors feel that part of the lack of agreement between the different methods could be explained by lack of precision of interpretation of the fetal monitor strip or assignment of the Apgar score?

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Dr. Divina J. Santos, Cincinnati, Ohio. Since 1953, when Virginia Apgar proposed the use of the now famous Apgar score, several advances have occurred in the practice of obstetrics; among them are electronic fetal and uterine monitoring and scalp and umbilical vessel sampling for pH and blood gas analysis. As a result of pregnancies being increasingly planned, wanted, and fewer in number, each pregnancy is more precious and there is greater expectation of a perfect outcome. Any deviation from the expected perfect result can lead to a medicolegal litigation. There is a long statute of limitation. A child up to the age of 21 can now sue the obstetrician for wrongful birth.

Through the years, the interpretation of the Apgar score has been expanded to include using it to predict occurrence of intrapartum birth asphyxia and subsequent long-term neurological outcome. Recently, however, many investigators have questioned the ability of the Apgar score to make these predictions. As a result, there is increasing use of intrapartum scalp sampling and umbilical blood analysis for pH and blood gas values. The estimation of base deficit allows differentiation between respiratory (acute asphyxia) and metabolic (chronic or long-standing asphyxia). The authors of this article attempted to find a correlation between the neonatal acid-base status, Apgar scores, and fetal heart rate tracing.

This article raises several questions:

1. Who attended the deliveries? Who performed the resuscitation? How many babies had meconium-stained amniotic fluid, and how many had a nuchal cord? Was there a relationship between the low Apgar scores, acidotic pH, and any of these variables?
2. Was there a higher incidence of elective outlet forceps in those that had an abnormal fetal tracing?
3. Was there a greater number of abnormal tracings in those that had hypotension during regional anesthesia?
4. What were the range and average length of fetal monitoring? Was the entire record examined? Of those 18 abnormal tracings, did the babies that also had low Apgar scores have a worse tracing than those that did not? Were the abnormalities in the tracing more pronounced in the last 30 to 60 minutes before delivery in those babies with low Apgar scores and pH <7.20?
5. Were there any medications given during labor that could affect the Apgar score, such as narcotics, tranquilizers, or magnesium sulfate?
6. Was the base deficit estimated in all cases? Did the babies with a pH <7.20 have metabolic or respiratory acidosis?

Table I. Mean differences from control (time 0) at 15 minutes (n = 14)

	Venous	*Arterial*
pH	−0.006	−0.022
P_{CO_2} (mm Hg)	0.143	0.628
P_{O_2} (mm Hg)	0.414	0.964

7. Of those infants that had a pH <7.20, how low were the Apgar scores?

8. What was the base deficit in those babies that had abnormal fetal heart rate tracings and those with low Apgar scores? Were those babies with large base deficits the same ones that had persistently low 5-minute Apgar scores?

Because the Apgar score does not always indicate presence or absence of intrapartum asphyxia, some institutions have adopted a policy of obtaining umbilical cord pH and blood gas values in selected cases or routinely in all deliveries. Since each determination costs approximately $25.00, it may not be cost effective to do this laboratory test for all deliveries.

We recently studied the stability of pH and blood gas values in a doubly clamped umbilical cord left at room temperature. We found that sampling of the umbilical artery and vein can be delayed up to 15 minutes without invalidating the values of the pH, P_{CO_2}, and P_{O_2} when compared with the values obtained from umbilical blood samples at birth. Table I shows the mean differences in pH, P_{CO_2}, and P_{O_2} of umbilical venous and arterial samples collected at 15 minutes when compared with pH and blood gas values obtained immediately at birth. There was no statistically significant difference noted when the results of samples collected at 15 minutes were compared with those of samples collected immediately after delivery.

In most instances, 15 minutes should be ample time to assess the newborn infant, assign the Apgar score, and decide if umbilical arterial pH and blood gas values will be necessary to elucidate some confusing clinical picture and establish the acid-base status at the time of delivery.

I agree with the authors' conclusion that we should utilize all three methods available in our armamentarium to assess the neonate, not only immediately at delivery but also prospectively and retrospectively, if we are to continue to improve the quality of care we render our patients and their babies.

Dr. Chang Y. Lee, Detroit, Michigan. We are faced with the necessity of reappraising the tools used for the diagnosis of fetal asphyxia. The important question asked by many investigators is does fetal asphyxia diagnosed by these tools indeed predict the development of neurological abnormalities? Since I am the third discussant assigned to this preliminary report, instead of dissecting the paper, I would like to extend my discussion to questions regarding fetal asphyxia and the development of neurological deficit.

In a prospective study of 1210 consecutive deliveries, Sykes et al.[1] found that only 21% of the neonates with a 1-minute Apgar score <7 and 19% of the neonates with a 5-minute Apgar score <7 had a severe acidosis (umbilical cord blood pH ≤7.10 and base deficit ≥13 mmol/L). The majority of neonates (86%) with severe acidosis had an Apgar score of 7 at 5 minutes. Silverman et al.[2] also found similar results with more than 1000 neonates. It is apparent in these studies that physical fetal depression (low Apgar scores) occurs with severe and prolonged asphyxia in the healthy fetus.

In a collaborative perinatal project, Nelson and Ellenberg[3] evaluated Apgar scores as predictors of chronic neurological disability. Among 390 infants with Apgar scores of 0 to 3 at 10 minutes or later, 270 infants died before their first birthday. Of the remaining 120 infants, 99 were followed up to 7 years of age; 87 were free of disabling cerebral palsy and only 12 (12%) children developed cerebral palsy. This study demonstrated that the outcome of this extreme asphyxia was either death or apparent intact survival.

After this study of 760 asphyxiated neonates as judged by Apgar scores and abnormal acid-base and blood gas status, Brown et al.[4] noted: "Asphyxia occurs in 54 per 1,000 live-born infants of whom only 7 are likely to be at risk of permanent brain damage. The dilemma of the obstetrician is that he has no way of knowing antenatally which are the 7."[5] We are, however, making every effort prenatally to identify these seven. In the study by Haesslein and Niswander,[6] among 80 term infants born after intrapartum asphyxia, neurological damage occurred in only 5 with risk factors of chronic intrauterine asphyxia including postdates (three) and intrauterine growth failure (two). There is mounting evidence that chronic asphyxia or repeated acute episodes of asphyxia superimposed on chronic asphyxia lead to permanent brain injury.

Low et al.[7] assessed causes of newborn encephalopathy. Neonatal respiratory complications and infections were found to be the causes of severe encephalopathy in 66% of newborn infants compared with 22% caused by fetal asphyxia. A normal range of acid-base status at umbilical cord blood sampling may protect us from litigious action in some cases. I strongly support the recommendation of obtaining cord blood for protection in the current medicolegal environment. I encourage the authors to continue their study with long-term follow-up.

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DR. JOHN L. DUHRING, Toledo, Ohio. I would like to ask the authors if any type of emergency, perhaps a profoundly defective baby or a depressed neonate or anything that would interfere with the collection of the cord blood specimen, affected their studies? I think Dr. Santos touched on this. I would like to hear their experience.

DR. JOHN GARBACIAK, Cleveland, Ohio. Will the authors state specifically what method they used for the pH determination and, if they can give reasons, why they chose the umbilical arterial blood as opposed to the umbilical vein?

DR. CHARLES A. WHITE, New Orleans, Louisiana. There was a multiplicity of questions raised by the formal discussants. My question is of relatively minor importance, but the authors mentioned scalp stimulation under fetal assessment. I wonder if the authors would amplify on that.

DR. JAMES MAURER, Phoenix, Arizona. While the presence of meconium may be somewhat nonspecific, I wonder if any of the acidotic babies were born without the presence of meconium.

DR. MARTIN (Closing). All of our deliveries at the University Medical Center at Mississippi were attended by the house staff. There were two private patients attended by faculty. In general these were normal deliveries and the infants were attended by obstetrics personnel for resuscitation. Pediatrics personnel were consulted if there was a problem.

Dr. Hage had questions about technical problems. We had no clotting or other methodologic problems. Our average charge to the patient is $37. I think that is reasonable. One could just obtain the arterial pH, but we believe the base excess, Po_2, and Pco_2 are important tools in the evaluation.

There was a concern about the private practitioner in a smaller hospital. If the infant is compromised, the odds are good that the pediatrician will be evaluating blood gases anyway, so we do not feel this would be any extra inconvenience for the laboratory personnel to analyze the cord blood sample.

Also, as far as maternal blood gases are concerned, we did not routinely determine those because we had normal mothers without asthma or any pulmonary disease or ketoacidosis. It would be advisable to obtain a maternal acid-base study if you suspect an abnormality.

In cases of fetal compromise, since most of the authors whose articles I have read have recommended immediate clamping of the umbilical cord for acid-base studies, I do not think that this would interfere with obtaining umbilical cord samples. We would obtain an umbilical arterial specimen, since it has been shown to correlate better with scalp pH. Also it makes more sense to sample blood coming from the fetus to the placenta as it will more adequately reflect fetal status than blood from the placenta returning to the fetus. Actually, I think there is a 0.1 higher difference in the pH.

The scalp stimulation test as described by Dr. Miller involves manipulating the fetal scalp and looking for a rise in fetal heart rate similar to that with the nonstress test. The specific method used was to put an Allis clamp on the scalp to see if the fetal heart rate acceleration occurred after that.

We had four infants with meconium. Three were normal; one had an abnormal fetal heart rate tracing. All had normal pH values. There were two infants with nuchal cords and both had normal fetal heart rates and Apgar scores. There were certain drugs administered that could affect the variability and the heart rate tracing. Demerol was given in small amounts, the maximum being three 25 mg doses in one patient. We did have three patients with pregnancy-induced hypertension. They received magnesium sulfate but also had normal acid-base studies in the fetus.

Dr. Hage pointed out that there is disagreement about the interpretation of the fetal heart rate tracings and Apgar scores. These are generally methods that most of us are using to evaluate the fetus. We are looking for more objective and less subjective means of evaluating the fetal status, and that supports our claim that umbilical cord studies should be done.

Dr. Santos made a good point, that we could delay obtaining the blood sample for up to 15 minutes after delivery. Thus we could go back and look at the cord blood gas values and assess our care, should a problem be detected in a baby after delivery.

Upper genital tract changes and infertility in diethylstilbestrol-exposed women

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A possible relationship of upper genital tract abnormalities in the diethylstilbestrol-exposed woman and infertility was studied. Six hundred thirty-two women had roentgenography performed. Three hundred sixty-seven of these women were not using contraception and were actively trying to become pregnant. One hundred thirty-four (36%) of these women reported difficulties conceiving for a period of 1 or more years. Seventy-three percent of these women had abnormal hysterosalpingograms. The frequency of abnormal x-ray films in the women who did conceive within 1 year was essentially the same (74%). Furthermore, a similar number of women with normal x-ray films (37%) had difficulty conceiving as did women with abnormal x-ray films (36%). When specific hysterosalpingographic abnormalities were related to the presence or absence of infertility, it was observed that the presence of a constriction of the upper uterine cavity resulted in a 2.26-times greater likelihood that a woman would not be able to conceive. If a T-shaped uterus was found in association with constriction of the upper uterine cavity, the odds ratio for inability to conceive was found to be 2.63. (Am J Obstet Gynecol 1986;154:1312-8.)

Key words: Diethylstilbestrol exposure, hysterosalpingogram, uterine abnormality, infertility

Pregnancy outcome in women exposed to diethylstilbestrol before birth is significantly worse than in the nonexposed patient. An association between upper genital tract abnormalities and this poor pregnancy outcome has been found.[1] There is disagreement, however, regarding the frequency of infertility in the diethylstilbestrol-exposed woman compared with the unexposed individual. Barnes et al.[2] have reported that infertility, which was measured in pregnancies achieved, did not differ between women exposed to diethylstilbestrol and a control population. Bibbo et al.[3] and Herbst et al.,[4] however, have noted an increased occurrence of infertility in the diethylstilbestrol-exposed woman. The present study was undertaken to investigate whether there is an association between structural changes of the upper genital tract and infertility. The answer to two questions was sought. First, is there an association between any upper genital tract abnormalities and the ability to conceive? Second, are specific structural changes of the upper genital tract related to infertility?

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Material and methods

The patients studied were selected from women who had been enrolled in a national study funded by the National Cancer Institute to identify and follow up women exposed in utero to diethylstilbestrol (Diethylstilbestrol Adenosis or DESAD Project). Patients were recruited from two of the centers participating in this program—Baylor College of Medicine, Houston, Texas, and the Mayo Clinic, Rochester, Minnesota. The details of patient recruitment and selection for the DESAD Project were presented earlier.[5] The details of patient recruitment for hysterosalpingography have also been published.[1]

The methods of obtaining maternal and patient history, as well as findings of the first examination,[6,7] have been reported in previous DESAD communications as has the technique of hysterosalpingography and uterine evaluation.[8] Before initiation of the study, the investigators from Baylor College of Medicine and the Mayo Clinic standardized the x-ray review in order to ensure that the evaluations were consistent. The majority of the x-ray films were reviewed only at the center where they were performed. The shape, size, and contour of the uterine cavity were evaluated. Uterine defects, contour and appearance of the cervix, and changes in the oviducts were also recorded. In particular, the following abnormal conditions were documented during review of the films: cavity shape as T-shape, arcuate, hypoplastic, wide lower segment, bicornuate, unicornuate, constriction of upper-middle-lower segment, cornual constriction; intrauterine de-

Table I. Comparison of sexual activity status and pregnancies by method of selection of study participants

	Baylor			
	Walk-in plus referral	*Record review*	*Mayo: Record review*	*Total*
No. of women evaluated	339	99	194	632
No. never sexually active	6	3	7	16
Sexually active				
Never pregnant	153	35	53	241
Ever pregnant	180 (54%)	61 (64%)	134 (72%)	375
No. reporting difficulty conceiving	119 (36%)	41 (43%)	54 (29%)	214

Table II. Outcome of hysterosalpingography

	Baylor				*Mayo: Record review*	
	Walk-in plus referral		*Record review*			
Hysterosalpingogram	*n*	%	*n*	%	*n*	%
Inadequate	54	16	16	16	35	18
Normal	63	19	40	40	48	25
Abnormal	222	65	43	44	111	57
Total	339	100	99	100	194	100

fects as synechiae, diverticula, filling defect, irregular margins, ridges; oviducts—constriction, irregular borders, loculation, shape, patent or nonpatent.

At the time of each annual follow-up visit, the patients completed a health-history questionnaire. This questionnaire included information relative to sexual activity, contraception, desire to become pregnant, difficulty in conceiving, and pregnancies achieved. The information regarding difficulty in conceiving, infertility, and workup for infertility was given by the patients and was not verified by physician's records.

The manner of selection of patients for enrollment in a study such as this can influence to a significant degree the findings noted, as has been clearly demonstrated in prior DESAD reports.[1, 5, 6] The patients entered into the hysterosalpingogram study were selected from each of the four participant sources, the most reliable and unbiased of which was from the record review category. At Baylor, 41% of the walk-in and referral and 28% of the record review participants volunteered for the hysterosalpingogram study. At Mayo, only record review participants were asked to volunteer, and 36% of these women agreed. However, in the attempt to evaluate the relationship of possible infertility to structural changes of the upper genital tract, it was felt that combining all of the groups (record review, walk-ins, and referrals) for this study would not introduce a significant selection bias. Furthermore, since the data for both Baylor and the Mayo Clinic were similar, the data from both of these institutions were also grouped together. There was some degree of self-selection bias since a higher percentage of women who volunteered for the hysterosalpingogram study had difficulties conceiving compared with those women who did not participate in this study.

Information regarding attempted and successful pregnancies was available for 632 of the women in whom hysterosalpingography was performed. Three hundred sixty-seven of these women were not using contraception and were actively trying to achieve pregnancy. Information relative to success in achieving pregnancy, difficulty achieving pregnancy, and inability to become pregnant was related to the hysterosalpingographic findings.

Statistical analyses were done by means of the χ^2 test and Haldane's methods for odds ratios and estimation of confidence limits.

Results

X-ray films of 632 women were reviewed as part of this study (Table I). Of these 632 women, 16 were not sexually active and 214 reported some difficulty in conceiving. Women of the walk-in and referral groups who participated in the hysterosalpingogram study, as expected, had a lower rate of pregnancies (54%) than women of the two record review groups (64% and 72%, respectively). Within the groups, there were no differences between women who had hysterosalpingograms and those who did not enter the study.

Table II presents the outcome of the hysterosalpingograms in the entire study group. Three hundred seventy-six x-ray films were reported as being abnormal (59%).

Three hundred sixty-seven of the 632 women were

Table III. Difficulty in conceiving in women who tried to achieve pregnancy

	Hysterosalpingography							
	Normal		*Abnormal*		*Total*		*Not done*	
	n	%	*n*	%	*n*	%	*n*	%
No. of women studied	97		270	74*	367		650	
Attempted pregnancy ≤1 yr								
Conceived	59	61†	167	74* 62†	226	62†	528	81†
Did not conceive	2	2†	5	2†	7	2†	5	1†
Had difficulty conceiving ≥1 yr	36	37†	98	73* 36†	134	36†	117	18†
Conceived	21	58‡	55	72* 56‡	76	57‡	88	75‡
Between 1 and 3 yr	16	76§	25	61* 45§	41	54§	61	69§
After 3 yr	5	24§	30	86 55§	35	46§	27	31§
Did not conceive	15	42‡	43	74* 44‡	58	43‡	29	25‡
Tried 1-3 yr	9	60‖	24	73* 56‖	33	57‖	18	62‖
Tried ≥3 yr	6	40‖	19	76* 44‖	25	43‖	11	38‖

*Percent of row.
†Percent of women studied.
‡Percent of women with difficulty conceiving.
§Percent of women with difficulty conceiving but did conceive.
‖Percent of women with difficulty conceiving and did not conceive.

Table IV. Types of findings in 270 hysterosalpingograms considered abnormal

*Abnormality**	*n*	%
T-shaped	134	50
Hypoplastic	36	13
Wide lower segment	49	18
Constriction		
Upper	73	27
Middle	25	9
Lower	29	11
Cornual	88	33
Irregular margins	108	40

*Seen singly or in combination with other abnormalities.

Table V. Odds ratio for inability to conceive for ≥1 year by types of hysterosalpingographic abnormality

Frequency (%)	*Hysterosalpingography*	*Odds ratio*	χ^2
	Abnormal*	1.02	
50	T-shaped uterus†	1.49	1.4
26	Constriction of upper cavity	2.26 1.1-4.7§	3.98
22	T-shaped uterus and constriction of upper cavity†	2.63 1.2-5.7§	5.34

*Includes any abnormality.
†Includes any other abnormality.
‡Includes any other abnormality except T-shaped uterus.
§Ninety-five percent confidence interval.

not using contraception and were actively trying to become pregnant. Table III demonstrates the data relative to their success in conceiving. One hundred thirty-four women (36%) were unable to conceive for a period of 1 or more years, yet only 18% of women who did not volunteer for hysterosalpingograms reported such difficulties ($p < 0.01$). Seventy-three percent of the women who did not conceive had abnormal x-ray films. The frequency of abnormal x-ray films in the women who conceived within 1 year was essentially the same (74%). Furthermore, in women who had difficulty conceiving, the number of women with normal x-ray films (37%) was similar to the number of women with abnormal x-ray films (36%).

Of the 134 women having difficulty conceiving, 76 (57%) eventually conceived, whereas 58 (43%) were not able to achieve pregnancy during a period of 1 or more years. Seventy-two percent of the women who eventually conceived had abnormal x-ray films, where 74% of the women who did not conceive also had abnormal hysterosalpingograms. Of the group of women who had difficulty in achieving pregnancy for a period of 1 or more years, 58% with normal x-ray films and 56% with abnormal x-ray films eventually conceived. However, of the women who conceived in spite of difficul-

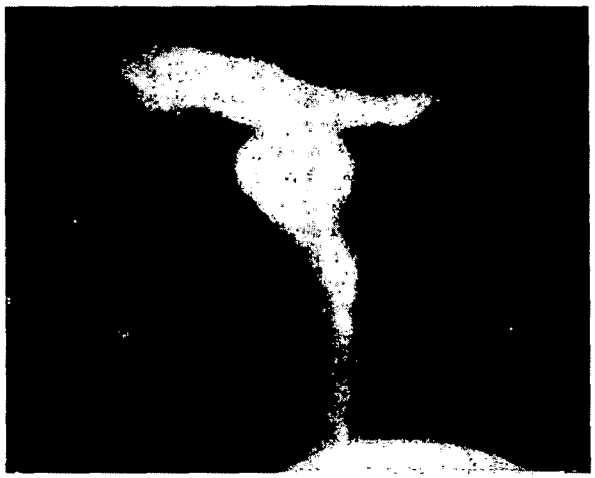


Fig. 1. Constriction of upper uterine cavity with T-shaped appearance of uterus.



Fig. 2. T-shaped appearance of uterus with slight constriction of upper uterine cavity.

ties, a higher number of those with normal x-ray films (76%) conceived within 1 to 3 years compared with those with an abnormal hysterosalpingogram (45%) ($p > 0.05$). Sixty-nine percent of women who did not volunteer for the hysterosalpingographic study and had trouble conceiving did so within 1 to 3 years.

Thus it would appear that the presence of a normal or abnormal hysterosalpingogram had no influence on the ability of a woman to achieve pregnancy. Furthermore, whether a woman conceived or did not conceive did not appear to be related to the presence or absence of an abnormal x-ray film.

Next, specific hysterosalpingographic abnormalities were compared to the presence or absence of infertility. Table IV demonstrates the types of x-ray abnormalities found in 270 women. Fifty percent of the x-ray films demonstrated a T-shaped appearance of the uterus. Irregular uterine margins, uterine constriction, and a wide lower segment were also commonly observed. Table V illustrates the odds ratio for inability to conceive of these 270 women relative to specific abnormalities noted in the uterus. The presence of an abnormal uterus per se did not increase the likelihood of a woman being infertile. This is also true for the presence of a T-shaped uterus. However, the presence of a constriction of the upper uterine cavity resulted in a 2.26 times greater likelihood of a woman not being able to conceive. If a T-shaped uterus was found in association with constriction of the upper uterine cavity (Figs. 1 and 2), the odds ratio for inability to conceive was found to be 2.63. Both of these latter figures are significant ($p < 0.05$). Other abnormalities or their combinations did not have any influence on ability to conceive (not tabulated).

The data relating to evaluation of infertility were relatively scanty in the records of our study population. One hundred seven women had been evaluated for infertility. Seventy-five percent of the women with normal x-ray films and 82% of the women with abnormal x-ray films had undergone an infertility workup. Thirteen of the couples were found to have a male factor related to infertility. An equal proportion of individuals with normal and abnormal x-ray films had husbands with abnormal semen analyses. Fifty-five women were found to have some factor not related to the hysterosalpingogram that was thought to contribute to the infertility. Here again, a similar percentage of women with abnormal and normal x-ray films was present in this group. In the latter group, 32 women eventually conceived. Equal numbers with normal and abnormal x-ray films conceived.

Comment

These data suggest that the presence of an abnormal hysterosalpingogram per se is not a factor contributing to infertility. Equal numbers of women with normal and abnormal hysterosalpingograms reported difficulty conceiving. Conversely, of those women having difficulty becoming pregnant the frequency of abnormal and normal x-ray films was equal. However, when specific abnormalities found on the hysterosalpingogram were evaluated, it was noted that the presence of a constriction in the upper uterine cavity did seem to be related to infertility in the population of women studied. The presence of a T-shaped uterus in association with constriction of the upper uterine cavity also appeared to increase the chances of infertility. The report by Berger and Alpel[9] suggested that infertility of

1 to 2 years' duration in patients with uterine deformities related to diethylstilbestrol exposure carried a poor prognosis for conception. However, they did not relate the infertility to specific uterine abnormalities because of the small number of patients in their study.

There are obviously several epidemiologic defects in this review. The definition of infertility was arbitrary. All women who had tried unsuccessfully for a period of 1 or more years to conceive were included in this category. Fifty-five percent of the women who had difficulty conceiving ultimately achieved a pregnancy after 3 years (30 of 55). Of those women who did not conceive, however, it is quite possible that some of these patients may have become pregnant after the termination of this study.

Another major weakness in this review is the lack of detailed data on the infertility evaluation of the study population. It is possible that factors other than uterine constriction may have been responsible for the greater frequency of infertility in this subgroup of patients. These data were not available on the questionnaire completed by the patients.

Having concluded that the presence of a uterine constriction may be associated with an increased risk for infertility, the obvious question arises as to what can be done to help such individuals to achieve pregnancy if they are unsuccessful in doing so. Surgical correction appears totally impractical and would probably result in greater uterine deformity than was initially present. This is an approach we would not currently recommend.

Of the women who had hysterosalpingography performed, 36% reported difficulties conceiving in contrast to only 18% of women who did not volunteer for x-ray studies. This suggests some self-selection bias. It is quite possible that some of the women with infertility problems elected to have the x-ray studies performed as part of their infertility workup. However, this should not have influenced the results obtained since the data compare the frequency of x-ray abnormalities relative only to infertility in women having x-ray studies performed. The data compare frequency of infertility in individuals with abnormal hysterosalpingograms as compared with women with normal hysterosalpingograms.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Rudi Ansbacher, Ann Arbor, Michigan. For the past 10 years, Dr. Kaufman has been interested in the genital tract changes in women exposed to diethylstilbestrol in utero. He and his associates first documented these changes in 1977,[1] and they include[2]: (1) T-shaped uterine cavity, (2) irregular contour of the uterine cavity, (3) hypoplastic uterus, (4) uterotubal constrictions, (5) cervical widening, (6) cervical-vaginal ridge, and (7) adenosis of the vaginal and cervical mucosa.

This study asked two questions: (1) Is there an association between any upper genital tract abnormalities and the ability to conceive? (2) Are specific structural changes of the upper genital tract related to infertility?

The authors pointed out the major inadequacies of their study, which include: (1) infertility was not defined specifically for their patient population; (2) the long-term follow-up of 3 years may have been inadequate; (3) there was a selection bias in the patient population studied.

Despite these inadequacies, some pertinent information can be derived from their data to answer the two questions they proposed. Women exposed to diethylstilbestrol in utero with abnormal hysterosalpingograms conceived at the same frequency as individuals who had normal x-ray films and, conversely, whether a woman conceived or did not conceive was not related to the presence or absence of an abnormal x-ray film. Therefore, in diethylstilbestrol-exposed women, it appeared that hysterosalpingograms depicting structural abnormalities of the uterine fundus played no role in the causation of infertility.

However, specific hysterosalpingographic abnormalities did appear to have a role in decreasing a woman's chance of conceiving if there was a constriction of the upper uterine cavity or if a T-shaped uterus was associated with constriction of the upper uterine cavity. There was a slightly greater than twofold likelihood of being unable to conceive in these women as compared to those with normal x-ray films.

Several questions must be asked of these authors.

They stated that a standardized x-ray review was used to ensure that the evaluations were consistent. Were the x-ray films exchanged between members of the two institutions for interpretation to ensure consistency in their interpretations?

Data relating to the evaluation of other factors associated with infertility were scanty. Were other factors that have a bearing on the success of achieving a pregnancy considered, including ovulatory dysfunction and the male factor? Why was not a properly defined control group compared with the women who were exposed to diethylstilbestrol in utero?

What was the age range of their subjects? Could this have been a factor in some of their subjects' inability to conceive? How do the authors define an inadequate hysterosalpingogram? Finally, do the structural changes, as described, affect the physiologic functions of the uterus, tubes, and cervix when compared with those of normal organs?

The authors have definitiely shown, in their defined study population, that specific hysterosalpingographic changes in diethylstilbestrol-exposed women are related to a decreased chance for fertility.

The authors are cognizant of the deficiencies in their study design, and I encourage them to further analyze their study population so that we, as clinicians, may properly counsel women who were exposed to diethylstilbestrol in utero and who desire fertility.

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DR. GITA R. GIDWANI, Cleveland, Ohio. In 1977 Kaufman et al.[1] published the study that showed abnormal hysterosalpingogram changes in 40 of 60 women exposed to diethylstilbestrol in utero. Since that publication, women with diethylstilbestrol exposure in utero have walked around with a label of "infertility handicap." We at the Cleveland Clinic did not obtain routine hysterosalpingograms on our diethylstilbestrol population. However, a hysterosalpingogram was obtained if such a patient was having a hysteroscopy or laparoscopy for bleeding, dysmenorrhea, pelvic pain, or workup of infertility in the operating room.

Then came the conflicting reports of various authors regarding fertility potential among diethylstilbestrol progeny. Thus, Herbst et al. and Bibbo et al. found significantly reduced fertility rates as compared with those of control subjects whereas Barnes and Cousins found no significant change. Berger, Goldstein, and Schmidt in uncontrolled series of patients also showed a decrease in the fertility of female diethylstilbestrol patients.[2] Even though various critics questioned the validity of the data because of the individualistic presentation of the data, it was difficult to reassure a diethylstilbestrol patient with an abnormal hysterosalpingogram that she would have no "reproductive handicap."

In July, 1984, the Norfolk group, in a small series of 20 diethylstilbestrol patients with abnormal hysterosalpingograms, showed that the fertility rate in these women was no different than that of the women with no diethylstilbestrol exposure.[3] Dr. Kaufman again in this article has shown that an abnormal hysterosalpingogram per se is not a factor contributing to infertility and that an equal number of women with normal and abnormal hysterosalpingograms reported difficulty in conceiving. Conversely, of the women having difficulty getting pregnant, the frequency of abnormal and normal x-ray films was equal.

There are several weaknesses in this study, for instance, selection bias; even though the data from Mayo and Baylor were grouped together, there was a higher percentage of women volunteering for the hysterosalpingogram study who had difficulty conceiving compared with those women who did not participate in the study. Similarly, the definition of infertility was arbitrary, that is, anyone unsuccessfully attempting conception for 1 or 2 years was included and women who may have conceived after the completion of the study were excluded. There was a lack of data on the availability of other factors in the evaluation of infertility. Of course, all the above deficiencies in the epidemiology of this study have been pointed out by Kaufman et al. in their discussion.

What we would like to know is the outcome of the pregnancies in the women with abnormal hysterosalpingograms. Thus the incidence of ectopic pregnancies, spontaneous abortions, and premature deliveries has been cited to be increased in such patients by other authors. Do we have information as to how many women had viable babies?

It seems from this article that presence of constriction in the upper uterine cavity did seem to be related to infertility and the presence of a T-shaped uterus in association with constriction of the upper uterine cavity also appeared to increase the chance of infertility. The question one would like to ask is did these pregnancies reach viability? Was an operation, for instance, cerclage, or use of other drugs, such as ritodrine, necessary in these pregnancies? This is important as we know that in uteri with different defects the reproductive performance is very much related to a particular shape of the uterus.

Perhaps in the future, reproductive centers with in vitro fertilization programs could address the above questions in their series of such patients with varying shapes of uteri so that more meaningful data on the functional efficiency of such uteri can be generated. We will then as clinicians be able to predict prospectively what the degree of "reproductive handicap" is in a particular patient exposed to diethylstilbestrol in utero.

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DR. EDUARD EICHNER, Cleveland, Ohio. I think the article is excellent. However, in our local area infertility includes the failure of live births, as Dr. Gidwani has stated, and the upper genital tract includes the cervix. Our biggest problem was cervical incompetence in these patients. If recognized early enough we were able to increase the positive results by cerclage, which Dr. Kaufman omitted from his study.

DR. SHAILAJA RAJ, New Orleans, Louisiana. Did the authors get any history of other drug(s) exposure (in utero) in the control subjects?

Second, do the authors have any opinion as to the cause of infertility in the group of women who had abnormality of uterus as the only cause?

DR. C. BRANDON CHENAULT, San Antonio, Texas. It was not clear to me in the discussion whether the abnormalities of the upper genital tract were changed by whether the patient was exposed to diethylstilbestrol or not. Would you clarify that for me, please?

DR. KAUFMAN (Closing). Both of the discussants very quickly noted some of the defects that we ourselves recognized in this article, but let me try to answer each of the questions as presented.

First, all the x-ray films were not reviewed by both the Mayo and Houston groups. Random x-ray films were looked at when we sent them back and forth in the mail or periodically when individuals from Baylor and Mayo met and reviewed a series of 20 to 25 x-ray films and compared their interpretations. We felt there was a relatively uniform interpretation of the x-ray films by both groups. Thus the reading of the x-ray films was fairly well standardized.

As far as the ages of the patients are concerned, I believe they varied between 19 and 32 years for individuals who had roentgenography performed.

We did not study a control population of women to look for factors causing infertility for the reason that we were simply looking to see if the presence of an abnormal x-ray film in our defined population increased the risk of infertility compared with that in women with normal x-ray films. The question we wanted to answer was whether the hysterosalpingogram would give us any hint as to the likelihood of infertility in the diethylstilbestrol-exposed woman.

The next question related to our defining an abnormal hysterosalpingogram. I believe this has been addressed in several prior publications. It was based on the appearance of the uterus: its contour, the size as estimated from the distance between the fundus and the internal os of the cervix, and the presence of various irregular defects within the uterus, such as filling defects, irregular margins, and diverticula.

The question was also raised as to how the structural changes affect the physiologic function of the uterus. I doubt very much if it is the shape of the uterus itself that affects its function. I suspect the change in the shape of the uterus is related to the functional capacity of the myometrium. There is probably a physiologic muscular dysfunction rather than a direct structural effect.

As far as the pregnancy outcome in this group of individuals is concerned, those data have also already been published. In general, the pregnancy outcome was very poor. Individuals with abnormal x-ray films had a much worse pregnancy outcome than those women with normal x-ray films. Overall, around 50% of pregnancies ended as term deliveries in women with abnormal x-ray films. Ectopic pregnancies occurred in between 8% and 10% of the individuals with abnormal x-ray films, and preterm delivery occurred in about 16% to 20% of this group of women. Even those individuals whose x-ray films were normal had a poorer pregnancy outcome than individuals who were not exposed to diethylstilbestrol, so the diethylstilbestrol exposure itself decreases the ability of these individuals to carry a pregnancy to term.

Another question was asked regarding the drugs that the patients' mothers had taken during pregnancy in addition to diethylstilbestrol. All of this material has been carefully looked into and has already been reported. The mothers had not taken any other drugs that should have been related in any way to the changes noted.

As far as whether the x-ray changes were related to the exposure to diethylstilbestrol, I do think the data would suggest that individuals who have been exposed to diethylstilbestrol in utero have a significantly higher likelihood of having uterine structural abnormalities compared with those women who have not been exposed. Obviously, unexposed individuals can occasionally show similar upper genital tract changes but with a frequency that is significantly less than in the exposed woman, probably in the range of 1% to 2%. In contrast, 40% of the diethylstilbestrol-exposed women in our record review population were found to have abnormalities.

Another question raised was related to the use of cervical cerclage in the pregnant diethylstilbestrol-exposed woman. We do not recommend that cerclage be performed except for standard indications—early dilatation and effacement of the cervix or a history of prior second-trimester abortion or early preterm delivery. In one of our previous publications, we reviewed the data on 17 diethylstilbestrol-exposed women who had cerclage performed during pregnancy. Cerclages had been done at random mainly because the patient had an abnormal x-ray film. The pregnancy outcome in this group of women was no better than that in women who did not have cerclage. Thus I would not recommend routine use of cerclage just because the woman has an abnormal x-ray film.

Primary carcinoma of the fallopian tube

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From 1964 through 1983, 47 patients with a mean age of 63 years underwent primary treatment of fallopian tube carcinoma. Vaginal bleeding, abdominopelvic pain or pressure, and a palpable pelvic mass (or masses) were frequent retrospective clinical associations. Survival analysis demonstrated a rapid initial rate of patient attrition with stabilization after 3 years, resulting in an overall 5-year survival rate of 41%. While independent of age, histologic grade, or original tumor size, patient longevity was significantly decreased ($p < 0.01$) by the presence of disease beyond the pelvis, neoplastic cells in the peritoneal washings, and size of residual disease. Therapy consisted of primary surgical resection, usually followed by adjunctive radiotherapy or chemotherapy. Initial sites of relapse were predominantly (88%) within the intraperitoneal cavity. The study supports definitive surgical staging and tumor reduction, as well as more selective adjuvant therapy based on surgical stage and residual tumor size and location. (Am J Obstet Gynecol 1986;154:1319-26.)

Key words: Fallopian tube carcinoma, survival analysis, staging, residual neoplasm, peritoneal cytology, relapse sites, treatment

Although the initial case of primary carcinoma of the fallopian tube was reported nearly a century ago,[1] the natural history and treatment of this disease process remain less well understood when compared with those of neoplasms of the adjacent müllerian structures. These uncertainties reflect at least in part the limited contemporary experiences at any single institution; few reports have witnessed the primary treatment of two or more patients per year during a reasonable study interval.[2-5] In addition, the variability in treatment, as well as the lack of standardized staging criteria, has resulted in suboptimal interinstitutional comparative evaluations. Our appreciation of the biologic aspects of this rare neoplasm and its responsiveness to currently available therapeutic technology will be accelerated through periodic reassessment by institutions or cooperative groups that treat larger patient populations.

We evaluated the clinicopathologic parameters of 47 patients treated for primary carcinoma of the fallopian tube and assessed the effectiveness of the treatment modalities used during the 20-year interval from 1964 through 1983.

Methods

The records of 47 patients with primary carcinoma of the fallopian tube who were treated at the Mayo Clinic between January 1, 1964, and December 31, 1983, were reviewed. The time of initiation of the present review coincided with the completion of a previous study (two decades) from our institution.[6] All lesions were retrospectively staged according to the criteria used for ovarian carcinoma, as suggested originally by Dodson et al.[7] At our institution, Stage III traditionally has been further subdivided into A and B, corresponding to residual disease ≤2 cm and residual disease >2 cm, respectively. Representative histologic sections of the primary lesions were available in all but one case and were reviewed by one of us (T. A. G.) who had no knowledge of the patient's clinical status. Both the histologic classification of Broders, as previously applied to fallopian tube carcinoma,[6] and the histologic classification suggested by Hu et al.[2] were employed for grading the neoplasms.

The following types of adjunctive radiotherapy were used: (1) intraperitoneal radioactive colloidal chromic phosphate P 32, (2) whole pelvic irradiation with or without para-aortic inclusion, and (3) whole abdominopelvic irradiation. Intraperitoneal colloidal chromic phosphate P 32 was administered at a dose of 15 mCi. The total doses, fractionation schedule, and field arrangement for both pelvic and whole abdominal irradiation were variable during the study interval. The total delivered dose for whole pelvic irradiation (with or without para-aortic treatment) ranged from 3500 to 5500 rad to the pelvis, with nine of 16 patients receiving 5000 rad or more. For patients treated with whole abdominopelvic irradiation, the whole abdomen (with partial shielding of the liver and kidneys when necessary) received from 1700 to 3500 rad, with additional doses to the pelvis and para-aortic regions in five of

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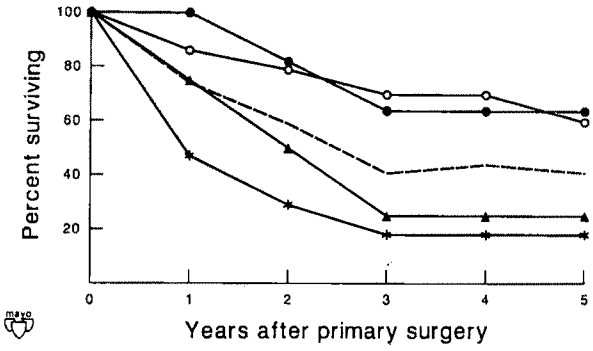


Fig. 1. Survival from time of primary operation according to tumor stage: ●——●, Stage I (n = 12); ○——○, Stage II (n = 14); *——*, Stage III (n = 17); ▲——▲, Stage IV (n = 4); - - - -, overall (n = 47).

seven patients, resulting in a total pelvic dose of 3800 to 5220 rad (four of five patients received additional doses to ≥4500 rad; one of five patients received additional doses only to 3500 rad).

Cyclophosphamide and L-phenylalanine mustard were used either alone or in the presence of progestational agents. Vincristine, cyclophosphamide, and either doxorubicin or dactinomycin were used initially (three patients) as combination therapy but more recently were replaced by cyclophosphamide and cisplatin (four patients).

To analyze the variable follow-up times (range, 15 months to 12 years) and censored survival data, the Kaplan-Meier method was used to estimate group survival. Tests of significance were based on log-rank tests for two-sample comparisons and logit-rank tests for continuous variables.

Results

The mean age of the 47 patients treated for primary carcinoma of the fallopian tube was 63 years. Evaluation of age by decade showed a peak frequency during the sixth and seventh decades; the youngest patient was 46 years old and the oldest was 84 years of age. Infertility was a common feature, with nulliparity noted in 40% (19 patients) and 57% (27 patients) having a parous status of ≤1. No relationship was noted between the associated infertility and venereal disease; only three of the 47 patients had a history of pelvic inflammatory disease, gonorrhea, and/or syphilis. Furthermore, no historical or concurrent evidence for either endometriosis or pelvic tuberculosis was found. However, in 10 patients, 12 other neoplasms were found before the tubal carcinoma was diagnosed. These included five breast, three colorectal, and two melanocytic lesions.

The most frequent presenting symptoms were vaginal bleeding (24 patients) and abdominopelvic pain or pressure (14 patients). In six patients, persistent postmenopausal bleeding eventually led to hysterectomy and the correct intraoperative diagnosis. Less frequently encountered presenting complaints were watery vaginal discharge, loss of weight, and neuropathy (three patients each). Seven patients had "other" symptoms or complaints.

Table I. Surgical stage and corresponding frequency of fallopian tube carcinoma (n = 47)

Stage	*Characteristics*	*No. of patients*
I	Carcinoma confined to fallopian tube(s)	12
A	Unilateral disease; no ascites	10
B	Bilateral disease; no ascites	0
C	Either A or B with ascites and/or neoplastic cells in peritoneal washings	2
II	Carcinoma extends beyond fallopian tube(s) but confined to pelvis	14
A	Extension to uterus and/or ovary	6
B	Extension to other pelvic organs	7
C	Either A or B with ascites and/or neoplastic cells in peritoneal washings	1
III	Carcinoma extends beyond pelvis but confined to abdominal cavity	17
A	Residual tumor size ≤2 cm	9
B	Residual tumor size >2 cm	8
IV	Carcinoma extends beyond abdominal cavity	4
Total		47

The cytologic assessment of 31 available cervical samples suggested neoplasia (Class V) in two patients (one also had a positive peritoneal cytologic study). In addition, the diagnosis of fallopian tube carcinoma was anticipated preoperatively in only three patients, two of whom had prior diagnostic procedures that identified the process. A palpable adnexal mass was present in 31 patients (66%), resulting in the clinicians generally suspecting an ovarian neoplasm. Nevertheless, while vaginal bleeding, abdominopelvic pain/pressure, and a palpable adnexal mass were frequently encountered, the complete triad occurred in only three patients (6%).

Primary surgical management consisting of staging and tumor resection was conducted at the Mayo Clinic in 36 patients (77%). The adnexal structures (either both or the remaining side) were excised in 45 patients, while hysterectomies were either previously or concomitantly performed in 41 patients. Omentectomy was accomplished in most patients, with 15% also requiring bowel resection. The distribution according to surgical stage is shown in Table I. Carcinomatous reduction to ≤1 cm was feasible in 31 patients (66%). Bilaterality was noted in four patients, with essentially equal distribution as to side in the remaining 43 patients. Involvement of the proximal third of the fallopian tube

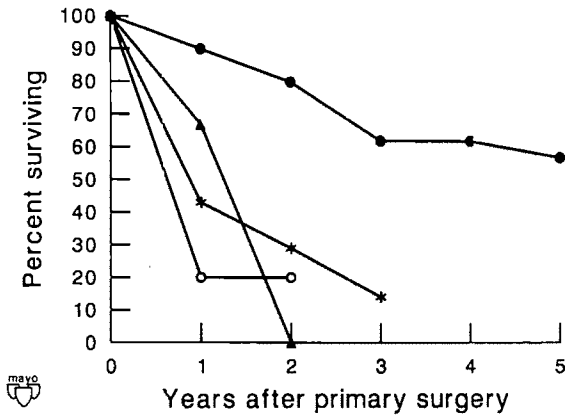


Fig. 2. Survival from time of primary operation to death from disease according to residual tumor volume: ●——●, <1 cm^3 (n = 31); ○——○, 1 to 10 cm^3 (n = 5); *——*, 10 to 100 cm^3 (n = 7); ▲——▲, >100 cm^3 (n = 3).

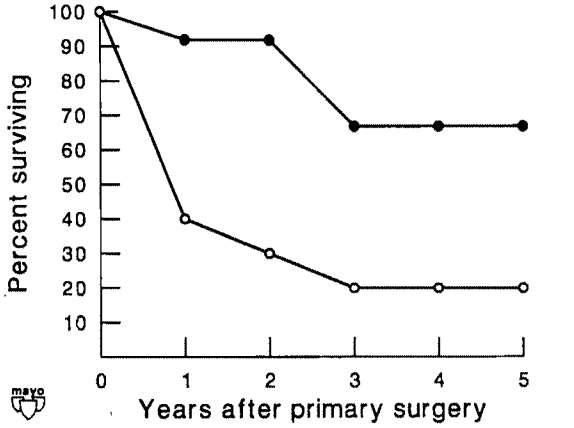


Fig. 3. Survival from time of primary operation to death from disease according to peritoneal cytologic findings: ●——●, Negative (n = 12); ○——○, positive (n = 10).

was not encountered among 37 patients eligible for site assessment. One patient had the tumor confined to the middle third only, whereas 22 had the distal third involved and 13 had the distal two thirds involved.

The overall 5-year survivorship was 41%, including a 26% attrition during the initial 12 months, with a continued decline to 44% at 3 years and stabilization thereafter (Fig. 1). While no significant differences were detected in longevity between patients having disease localized to one or both fallopian tubes (Stage I) compared with patients with disease beyond the fallopian tube but confined to the pelvis (Stage II), both groups fared substantially better ($p < 0.01$) than patients with carcinomatous involvement beyond the pelvis (Stages III and IV). Evaluation of estimated tumor volume before tumor reductive surgical procedures failed to identify a critical volume. However, survival rates for patients with residual tumor <1 cm in diameter were 80% and 57% at 2 and 5 years, respectively, and were superior ($p < 0.01$) to those of patients with larger residual tumor burdens (Fig. 2). Only three of the eligible patients in the latter subgroup were alive at 2 years.

Histologic assessment identified 43 adenocarcinomas, two squamous cell adenocarcinomas, and two mixed müllerian tumors containing adenocarcinomatous components. Both histologic grade and structural patterns were assessed. Survival at 5 years was 50%, 42%, and 39% for patients with papillary, papillary-alveolar, and alveolar-medullary patterns, respectively (p = NS). Likewise, overall 5-year survival rates were 44%, 37%, and 40% for patients with Broders' grades 1 and 2 (n = 11), 3 (n = 17), and 4 (n = 19), respectively. Individual analysis of survival according to grade within Stages I/II and III/IV also failed to demonstrate significant differences. In addition, assessment of histologic grade among patients with residual tumors of <1 cm suggested that well-differentiated lesions were associated with a more favorable survival relative to grade 3 lesions ($p = 0.03$) but not relative to grade 4 lesions (p = NS).

Peritoneal fluid or washings were obtained for cytologic analysis in 22 of 47 cases. The presence of exfoliated cells within the peritoneal cavity correlated with patient longevity (Fig. 3); survival at 2 and 5 years was 92% and 67% for patients with negative cytologic findings and 30% and 20% for those with positive cytologic findings, respectively ($p < 0.01$). As expected, intermediate survival rates of 56% and 39% were noted at 2 and 5 years for the 25 patients in whom cytologic samples were not evaluated.

Postoperative adjuvant therapy was routinely given and its impact assessed according to two subgroups (Table II): (1) patients with localized disease having complete removal with hysterectomy and bilateral salpingo-oophorectomy (Stages IA and IIA) and (2) patients with disease extending beyond the internal genitalia (Stages IC and ≥IIB). Among the 16 patients with localized disease, six died of disease (38%) at a mean time after operation of 30 months (range, 9 to 62 months). All but one of the deaths and five of six relapses (mean recurrence time, 13.5 months) occurred among the 10 patients who received no adjuvant therapy (two patients) or adjuvant therapy limited to the pelvis (three patients). However, five of the six patients subjected to more extensive therapy, with treatment of the upper abdomen by either whole abdominal irradiation or systemic cytotoxic agents in the presence or absence of intraperitoneal radioisotopes, remained relapse-free at a mean follow-up time of 39 months. The

Table II. Frequency and effectiveness of postoperative adjuvant therapy according to surgical stage

Stage	Type of adjuvant therapy*	No. of patients	Dead of disease		Progression-free survival (mo)	
			n	%	Mean	Range
Stages IA (n = 10) and IIA (n = 6)	None	5	2	40	60	9-102
	Chemotherapy, radiation	1	0		19	
	WPI†	6	4	67†	39	5-75
	WAPI	2	0		20	12-28
	^{32}P + M	2	0		68	49-86
Subtotal		16	6	38	46	
Stages IC and ≥IIB	None	3	3	100	30	4-54
	Chemotherapy, radiation	13	10	77	14	2-31
	WPI†	10	6	60†	46	6-140
	WAPI	5	5	100	13	5-62
Subtotal		31	24	77	27	

*WPI, Whole pelvic irradiation; WAPI, whole abdominopelvic irradiation; ^{32}P + M, radioactive phosphorus and L-phenylalanine mustard.

†One patient also received L-phenylalanine mustard.

only relapse and death in this subgroup occurred at 57 and 62 months, respectively, in a patient with Stage IIA disease who received L-phenylalanine mustard and pelvic irradiation.

In contrast, 24 of the 31 patients (77%) with disease beyond the pelvic genitalia died of disease despite adjunctive therapy. Fifteen patients had external irradiation; four of the 15 originally had presented with Stage IC, IIB, IIC, and IIIB disease and were relapse-free at 39, 140, 87, and 71 months, respectively, after receiving whole pelvic treatment either with or without para-aortic irradiation. Whole abdominopelvic irradiation was used in five patients, with two diagnosed as having Stage IIB disease (residual <1 cm) or Stage IIIA disease (residual <1 cm) and three with Stage IIIB disease, resulting in a mean time to progression of 18 months (median, 7 months), and all patients eventually died of disease. Systemic cytotoxic therapy was predominantly used as adjunctive therapy in patients with advanced stages. Initially, single alkylating agents were used either in the presence or in the absence of progestational agents; all patients so treated died within 15 months of the commencement of therapy. Combination chemotherapy subsequently replaced single-agent therapy, and longevity was marginally improved. Most recently, four patients received cyclophosphamide and cisplatin: two patients with Stages IIB and IIIB disease had progression of disease at 12 months, with death from disease occurring at 17 months in one patient and 18 months in the other, while two patients with Stage IIIA disease both were alive and free of progression at 31 months.

Progression or recurrence was experienced by 31 patients, with 61% and 84% of the relapses occurring within 12 and 24 months from the time of the primary operation, respectively; only two relapses were observed after 5 years (62 and 65 months). Among the 30 patients who died of disease were two in whom the specific cause of death was not ascertainable and four in whom the specific site of recurrence was not known. The initial sites of treatment failure were known in 24 patients, with the intraperitoneal abdominal cavity identified as the site of failure in 21 patients (88%).

Simultaneous metastastic involvement of single or multiple extra-abdominal organs also was found in six of the 24 patients, including the liver, nodes, vagina, lungs, and skin; some of these patients had involvement of more than one organ. Vertebral metastasis was the initial site of recurrence in three patients. Only one of the 24 patients survived free of disease—29 months from the time of documentation of relapse. Carcinoma at a "second-look" laparotomy was detected in a biopsy specimen of the abdominal peritoneum after the administration of 12 cycles of cyclophosphamide, vincristine, and dactinomycin for Stage IC disease. Whole abdominopelvic irradiation was given for microscopic persistence. Twenty of the remaining 23 patients died of disease within 18 months. The final three patients originally diagnosed as having Stage IIB disease and treated secondarily for recurrent disease with pelvic irradiation (operation alone originally), combination chemotherapy (operation plus whole abdominopelvic irradiation originally), and wide surgical excision (operation alone originally) died, one at 19 months, another at 26 months, and the third at 61 months after relapse.

Comment

Because the reported average annual incidence of carcinoma of the uterine tube is three per 1000 genital cancers,[6] the natural history and treatment of this disease remain less well defined than those of neoplasms

of the adjacent müllerian derivatives. The demographic features of the patients in our study were generally consistent with those from previous reports summarizing the literature.[2-4] While venereal disease, endometriosis, and tuberculosis may occasionally have been implicated in the pathogenesis of this neoplasm, historical or concurrent evidence for these processes in our review was infrequently obtained. These observations, together with the contrasting prevalence rates for pelvic inflammatory disease or endometriosis and fallopian tube carcinoma, further minimize the potential etiologic roles of these processes. However, circumstantial evidence for alterations in immunologic competence or tolerance in our and other series[8-10] is the number of patients with a prior or concomitant secondary malignant lesion.

Because carcinoma of the fallopian tube generally is manifested early by abnormal vaginal bleeding or abdominopelvic pain or pressure, confinement of disease to the pelvis at the time of diagnosis in most (55%) of our patients was therefore not unexpected.[3, 11, 12] Presumably, distention of the fallopian tube by the tumor or the resulting hydrosalpinx accounts for the frequency (30%) of associated pain. Although no involvement of the proximal portion of the tube was noted, it undoubtedly served as a conduit for blood and debris liberated by the more distal lesion and eventually was seen as abnormal vaginal bleeding in 51% of the patients. In addition, such an indirect portal to the neoplasm should imply value in cytologic sampling, which has been reported as positive in as many as 60% of evaluated patients.[3] Unfortunately, numerous reviews,[4, 7, 8, 11, 13] including the present and a previous report from our institution,[6] have been less successful in securing cytologic atypia, possibly reflecting suboptimal sampling. Despite the nonspecificity of the above signs and symptoms and the rarity of fallopian tube carcinoma, the presence of unexplained cervical or vaginal cytologic atypia, persistent unexplained abnormal vaginal bleeding or discharge after dilatation and curettage, persistent lower abdominal or pelvic pain or pressure, and the presence of an adnexal mass (66% of patients had a palpable mass in the present series) should prompt further investigation, with consideration given to diagnostic laparoscopy or laparotomy (or both) as indicated.[4]

The modes of dissemination of fallopian tube carcinoma are similar to those for ovarian cancer, occurring predominantly by cellular exfoliation with intraperitoneal spread and by lymphatic embolization to extraperitoneal sites. The process of cellular exfoliation is aided by the ready access of neoplastic cells into the coelomic cavity from their locations in the distal portion of the tube (95% in the distal one third or two thirds in our study).[8, 14] Also the sites of initial spread, including the omentum, peritoneum, intestines, and other pelvic genitalia, are similar to those described in ovarian carcinoma.[9, 13-15] These assumptions are further supported by the observation that 45% of the peritoneal washings analyzed in the present review contained neoplastic cells. In addition, a common site of metastatic infiltration at the time of documented recurrence or autopsy was the lymphatic system.[4, 9, 15] Tamimi and Figge[9] suggested that para-aortic nodal involvement is frequently encountered despite no evidence of intraperitoneal disease beyond the tube.

Because of the similarities of fallopian tube cancer to ovarian cancer, particularly in routes of initial spread and sites of subsequent recurrence, we favor the proposal of Dodson et al.,[7] employing staging criteria for fallopian tube carcinoma similar to those adopted for the ovary. However, the unusually large number of patients with disease confined to the pelvis (55%) and the similar 5-year survival rates for patients with Stages I (64%) and II (60%) disease relative to ovarian cancer suggest, at least in part, inadequate surgical staging, a fact readily apparent during the initial decade of our review. Other series with study intervals that began a decade or more before ours have noted Stages I and II disease in at least 70% of evaluable patients.[8, 11, 12] Hence the need for meticulous surgical staging as employed in ovarian cancer is apparent and should involve total abdominal hysterectomy, bilateral salpingo-oophorectomy, appendectomy, omentectomy, cytologic assessment of peritoneal washings, pelvic and para-aortic node sampling, and multiple peritoneal biopsies from high-yield areas, as dictated by the extent of gross disease.

Patient longevity was significantly decreased by the presence of disease outside the pelvis, the presence of neoplastic cells in the peritoneal washings, and the amount of residual disease at completion of the primary operation. The overall survival was 41% at 5 years, with nearly all deaths from disease occurring during the initial 36-month interval from diagnosis, undoubtedly reflecting inadequate or ineffective primary therapy. As noted above, no significant differences in 5-year survival rates were detected between patients with Stage I (64%) or II (60%) disease, but collectively this group fared better than patients with disease extending beyond the pelvis (Stage III, 18%; Stage IV, 25%). Furthermore, the results of peritoneal cytologic study seemed to have significant prognostic value, as patients who presented with negative peritoneal washings had a 3-year survival rate (67%) that was considerably more favorable than that of those patients with positive washings (20%). These observations again reinforce the necessity for inclusion of cytologic assessment in the definitive surgical staging procedure and treatment planning. Neither histologic grade nor size of the original

tumor burden seemed to have predictive significance regarding patient longevity, which is consistent with previous observations.[1, 6, 16] However, long-term survival was influenced by the size of the residual tumor after the primary operation, with a rapid attrition when residual tumor size was ≥1 cm. These results suggest that, in the overall therapeutic approach to this disease process, maximal tumor reductive efforts be made at the primary operation.

While advocates generally cite, in support of postoperative adjuvant therapy, high relapse rates among patients with localized disease and minimal salvage rates among patients with more advanced disease, the efficacy of such therapy is difficult to assess from the literature. The former, at least in part, reflects inadequate surgical staging and requires additional maturation and confirmation of treatment results after definitive intraoperative and pathologic assessment of the extent of disease before the role of adjuvant therapy in this group can be realized. In advanced disease, the various treatment modalities selected and endorsed as adjunctive therapy are generally based on limited successes in small samples and without regard to selection biases. Also the cytotoxic agents and specific dosages used, as well as the treatment fields, fractionations, and total dosages used in radiotherapy, varied considerably within the small series and among institutions. The present study is liable to similar criticisms; specifically, optimal staging was not performed during the initial decade of this review, and treatment selection frequently was determined by extent of metastatic involvement. Therefore, to reduce the impact of these deficiencies, this study population was subdivided according to the extent of disease. Patients having disease localized to the pelvic genitalia (Stages IA/B and IIA) and hence removed by surgical excision were evaluated with reference to the merits of postoperative adjuvant therapy, while patients with disease not amenable to surgical treatment alone (Stages IC and ≥IIB) were assessed for evidence that might lead to more appropriate treatment selection.

Disease localized to the pelvic genitalia (10 patients with Stage IA disease, six with Stage IIA disease) was assessed according to whether treatment was directed to the pelvis only or beyond the true pelvis. The group subjected to operation alone or supplemented with pelvic irradiation experienced a 50% relapse rate (five of 10 patients), with all patients eventually dying of disease, which is consistent with previous reports that addressed the value of adjunctive therapy in early stages.[6, 11, 14, 15] In contrast, among the patients having systemic or whole abdominal therapy or both, only one of six patients had recurrence, with the sole relapse to date occurring in the patient treated with L-phenylalanine mustard and pelvic irradiation. While limited sample size mandates cautious interpretation, these findings, as well as the predominant intra-abdominal failure patterns, support the use of postoperative adjuvant radiotherapy to the entire abdomen in this subgroup. Recently, Brown et al.[8] reported the use of postoperative whole abdominopelvic irradiation in three patients with Stage I/II disease and noted similar results, while Benedet et al.[13] also successfully treated two patients without macroscopic disease by use of intraperitoneal colloidal chromic phosphate P 32. From the available information, both the radiocolloid (chromic phosphate P 32) and external beam irradiation seem to be similarly effective in patients with Stages IA and IB and IIA disease, and both treatments merit further investigation, as has been previously suggested.[4, 8, 13]

The optimal therapy for patients with either microscopic or macroscopic residual disease beyond the pelvis remains ill-defined. Roberts and Lifshitz,[11] as well as a previous report from our institution,[6] questioned the merits of postoperative pelvic irradiation in patients with advanced disease. Consistent was the observation that only one patient with Stage III disease in the present series who received postoperative irradiation (pelvic and para-aortic) remains relapse-free, having had para-aortic nodal infiltration as the only extratubal site of metastasis. More recently, whole abdominopelvic irradiation was used in five patients who had extragenital spread, and progressive disease was noted within 7 months among three patients who had residual tumors exceeding 2 cm and at 12 and 62 months in two patients who had residual tumor of <1 cm. Recently, Brown et al.[8] reported that two of three patients with Stage III disease died within one year after whole abdominopelvic irradiation despite having no residual disease. Therefore, while the role of postoperative irradiation in the treatment of Stage IIB disease or greater of the fallopian tube appears limited, we continue to use whole abdominopelvic irradiation but only in patients with microscopic intraperitoneal disease beyond the pelvis or with minimal (<1 cm) residual disease within the pelvis or para-aortic sites.

As Yoonessi's original literature review[1] and the recent update by Brown et al.[8] indicate, experience with postoperative adjuvant chemotherapy for this tumor is likewise very limited. All patients in our series, irrespective of residual volume, who had disease beyond the genitalia and who received single alkylating agents, either in the presence or in the absence of progestational agents, died of disease within 15 months, which is consistent with the previous reports of only occasional responses, usually of short duration.[4, 5, 15] Deppe et al.[17] were the first to report the successful use of adjuvant combination chemotherapy involving cisplatin. Several subsequent reports,[5, 12, 15] including the present series

(two of four patients free of disease at 31 months) and the M. D. Anderson review (three of three patients free of disease at 9 to 33 months), suggest that regimens containing cisplatin may be of potential value in the treatment of advanced as well as recurrent fallopian tube carcinoma.

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Discussion

DR. GEORGE T. SCHNEIDER, New Orleans, Louisiana. As Dr. Podratz and associates have pointed out in this well-studied series of primary tubal carcinoma, this site of cancer in the woman is still one that resists early detection and a good chance of a complete cure. This is the bad news. The only good news, I guess, is that the incidence of tubal carcinoma is still <1% of all gynecologic malignancies.

As with carcinoma elsewhere, the prognosis for fallopian tube carcinoma is determined by the anatomic extent and the grading of the disease. The authors report an overall 41% survival for 5 years, but if all of the tumor could be removed, as in Stage I and Stage II, a 64% survival rate was obtained. These figures are comparable to those of most other reported series and are quite similar to a 40% overall cure rate in our cases reviewed recently at our institution (Ochsner Foundation Hospital).

The big problem, of course, is that early detection of this lesion is infrequent and the incidence of a correct preoperative diagnosis is very low. For example, in this series of 47 cases only one was diagnosed correctly, and in the recent M. D. Anderson series, only two of 71 cases were diagnosed correctly preoperatively. This is due to the extreme rarity of fallopian tube carcinoma as compared with the more naturally suspected lesion of ovarian carcinoma.

As with endometrial and ovarian carcinoma, peritoneal cell washings should be obtained in all patients operated on with endometrial and ovarian carcinoma because favorable prognosis is directly related to negative cell findings. The addition of intraperitoneal chromic phosphate P 32 should be considered in selected cases where the tumor is resectable but cell washings are positive.

When one opens the abdomen to find an extensive, unexpected tuboovarian carcinoma, adequate excisional biopsies for immediate in vitro cultures should be obtained. There are a number of good laboratories now around the country where appropriate chemotherapeutic agents can be tested in vitro, and, therefore, a more appropriate chemotherapeutic regimen can be given.

It is disappointing that Dr. Podratz cannot give us much prognostic help with adjunctive therapy such as radiation and chemotherapy, and this is certainly true with other series. Hopefully, less toxic and more efficient chemotherapeutic agents may be developed in the future, and the use of intraperitoneal intraoperative direct radiation at the time of operation may be helpful as the technique of this radiation therapy is improved. Our surgical group in New Orleans has used it in approximately 25 cases other than pelvic cancer, mainly involving the pancreas, colon, and brain.

Finally, I would like to describe an interesting case of primary tubal carcinoma that is extremely rare; the epithelial type was squamous cell carcinoma in situ. This was in a 50-year-old patient who 10 years previously had undergone total hysterectomy and left salpingo-oophorectomy elsewhere for carcinoma in situ of the cervix. She presented with a right adnexal mass, which at operation proved to be a benign cystadenoma, but the adjacent tube removed routinely showed an epidermoid carcinoma in situ found incidentally in the pathologic specimen. The ampullary portions of the tube were free of neoplasm. We could find only two reported cases of possible primary epidermoid carcinoma of the uterine tube in the English literature. This illustrates the multicentric origin of squamous neoplasia in the müllerian system in the woman. It is interesting that 1 year after operation a vaginal vault dysplasia in this patient was detected and treated. She is

now being followed up carefully to detect any subsequent vulvo vaginal dysplastic changes.

Dr. Joseph C. Scott, Omaha, Nebraska. In patients with positive cytologic test results or those who had evidence of residual disease, did supraclavicular node biopsy play any part in deciding the future management of the patients?

Dr. Alexander W. Kennedy, Cleveland, Ohio. I would like to ask the authors two questions, one in regard to carcinosarcomas or sarcomas of the fallopian tube. We have encountered several of these recently, and I would like to know if they have encountered any in their review.

Second, did any of the patients have estrogen- or progesterone-binding protein determined?

Dr. David Hoogerland, Milwaukee, Wisconsin. Did any of the patients have periaortic lymph node involvement in the absence of any other evidence of spread?

Dr. Podratz (Closing). Dr. Schneider's sharing his interesting case again illustrated the association of secondary malignancies and possible alterations in the immune mechanisms of these patients.

With regard to Dr. Masterson's inquiries pertaining to peritoneal cytology, peritoneal fluid or washings were obtained on entry into the abdominal cavity before exploration. The pelvic viscera and the right hemidiaphragm, liver, and right upper colic gutter were washed with saline solution and the two aspirated samples were submitted for centrifugation and cytologic assessment of the resulting pellet. No attempt was made to quantitate the number of neoplastic cells.

Metastatic periaortic involvement is occasionally encountered in the absence of intra-abdominal disease and recently has been addressed in detail by Tamimi and Figge (1981). Particularly with complete agglutination of the distal end of the fallopian tube, the rich lymphatic network would predispose for this setting. Such patients with surgical Stage III disease made up a small subset in this study with one long-term survivor noted after whole pelvic and periaortic irradiation. Scalene nodes were not routinely sampled in patients with positive peritoneal washings or periaortic nodes. Our experience with this diagnostic technique has been limited to the evaluation of the patients with advanced cervical carcinoma. Likewise, intraoperative irradiation, while occasionally incorporated in the overall therapeutic approach for advanced cervical carcinoma at our institution, has not been used in the treatment schema of fallopian tube carcinoma to date.

This review was limited to adenocarcinoma of the fallopian tube but did include two cases of mixed müllerian tumors. Both contained an adenocarcinomatous component and therefore were included. In addition, a limited number of lesions were submitted for estrogen and progesterone receptor analysis. Considering the study time interval, the number of such analyses was not large enough to warrant interpretation.

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Immunoglobulin G subclasses and isoimmunized pregnancy outcome

M. Mark Taslimi, M.D., Baha M. Sibai, M.D., James M. Mason, Ph.D., and John V. Dacus, M.D.

Memphis, Tennessee

Immunoglobulin G antibodies are the most frequent type of red blood cell isoantibodies. They possess the ability to traverse the placenta and may cause hemolytic disease in newborn infants. Of the four subclasses, IgG1 and IgG3 are effective at binding erythrocytes to monocyte-macrophages and therefore are potentially destructive to erythrocytes. IgG2 and IgG4 are unable to react effectively with receptor sites on phagocytic cells and so are totally inactive relative to hemolysis. IgG subclasses were identified in 22 consecutive isoimmunized antepartum patients. Fifteen patients were subsequently delivered of babies who were either affected by or susceptible to hemolytic disease of the newborn. When maternal antibodies consisted of IgG1 only, hemolytic disease of the newborn developed in four of five cases. When only IgG3 was present, each of two patients developed hemolytic disease of the newborn. In eight cases both IgG1 and IgG3 were present and all the babies were born with hemolytic disease of the newborn. Determination of IgG subclasses might prove to be a useful adjunct in evaluating isoimmunized pregnancies. However, the limited information available does not permit the use of IgG subclasses exclusive of traditional means of diagnosis and management of isoimmunized pregnancies. (Am J Obstet Gynecol 1986;154:1327-32.)

Key words: IgG subclasses, isoimmunization, hemolytic disease of newborn

The incidence of Rh isoimmunization is declining. However, isoimmunization with irregular antibodies still occurs, mostly as a result of blood transfusion,[1] and isoimmunization of pregnant women can occur as a result of antepartum or intrapartum fetomaternal hemorrhage during spontaneous or elective abortion, as a result of amniocentesis, or in the course of ectopic pregnancies.[2] As a result, hemolytic disease of the newborn is still a continuing problem.[3, 4] Immunoglobulin G antibodies are the most frequent class of red blood cell antibodies[5] and are actively transported across the placenta.[6] However, occurrence or severity of fetal and neonatal hemolysis does not correlate well with the titer of circulating maternal antibodies.[7]

Chemical structure and biologic behavior of IgG subclasses of blood group antibodies have been studied by biologists and immunologists during the past two decades.[5, 8] However, the findings have not been widely applied to the care of isoimmunized pregnant patients. IgG antibodies are divided into four subclasses based on their structural and chemical properties: IgG1, IgG2, IgG3, or IgG4. Presence of IgG antibodies on red blood cells per se does not significantly affect red blood cell survival.[5] However, antibody-coated erythrocytes, on reaching the spleen, adhere to macrophages and hemolysis occurs.[9] IgG1 and IgG3 can react with specific receptor sites on phagocytic cells and so are potentially destructive to red blood cells. IgG2 and IgG4 are totally inactive in this regard.[5, 8, 10] The purpose of the present study was to investigate the association of maternal IgG subclasses with the development of fetal and neonatal hemolysis in 22 pregnancies complicated by isoimmunization.

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Material and methods

Between January 1, 1982, and December 20, 1984, we studied 22 consecutive pregnancies that had clinically significant blood group antibodies and that had been referred to the University of Tennessee Center for the Health Sciences. Maternal serum from all patients was screened for blood group antibodies according to techniques and a protocol outlined in a previous report.[11] Patients with positive indirect Coombs tests were evaluated by periodic antibody titer, serial ultrasonography, and spectrophotometric analysis of amniotic fluid bilirubin. Decisions for fetal intrauterine blood transfusion and interruption of pregnancy were made on the basis of these evaluations. IgG subclasses were identified in all patients. When serial antibody and subclass determination and amniocentesis were performed, the last test before delivery was used as the reference. Cord blood samples were collected and

Table I. Laboratory findings and pregnancy outcomes in 22 pregnancies complicated by isoimmunization

Patient No.	Antibody identified	Indirect Coombs titer	IgG subclass	ΔOD_{450} (zone)	Cord blood			Neonatal weight (gm)	Pregnancy outcome, HDN
					Direct Coombs titer	Antibody eluted	Hematocrit (%)		
1	D	1:256	1,3,4	III	NA	NA	25	2600	Severe, IUT
2	D	1:512	1,3	III	NA	NA	30	1380	Severe, IUT
3	D	1:1024	1,3	III	NA	NA	22	2080	Severe, IUT
4	D	1:256	1,3,4	II	+	D	35 28	960 1870	Twin 1, moderate Twin 2, severe
5	D	1:256	1,3	I	+	D	39.9	3320	Mild
6	D	1:512	1,3	II	+	D	34.8	2240	Mild
7	D	1:256	3,4	ND	+	D	38	2296	Mild
8	D	1:128	1,3	I	+	D	51.6	3720	Mild
9	Kp^b	1:256	1,2,4	III	+	Kp^b	21	2030	Moderate
10	Kell Jk^a	1:512 1:4	1,2,3 2	II	+	Kell Jk^a	66	2783	Mild
11	D Js^b	1:128 1:32	1,4 4	I	+	Js^b	41	3550	None
12	U	1:8	3	II	+	ND	54.8	3310	Mild
13	Js^b	1:2048	1	ND	+	Js^b	49.5	2210	None
14	S	1:256	1	I	+	S	60	3110	None
15	Fy^a	1:32	1	I	+	Fy^a	62	4040	None
16	Kell	1:256	1,2	I	Neg.	NA	—	2940	None
17	Kell	1:1024	1	I	Neg.	NA	57	2110	None
18	Js^b	1:32	1	ND	Neg.	NA	—	2600	None
19	S	1:32	1,2	I	ND	ND	62.5	2350	Delivered elsewhere
20	Kell	1:512	1,2	ND	ND	ND	58	3290	None
21	U	1:64	1,3,4	ND	Neg.	ND	—	3780	None
22	Kell	1:16	1,2,3	ND	Neg.	NA	63	2860	None

NA = Not applicable, HDN = hemolytic disease of newborn; IUT = intrauterine transfusion; ND = not done.

tested as detailed previously,[11] and the type of antibody eluted from fetal erythrocytes was determined. Infants with positive cord blood antiglobulin tests were monitored by serial determination of serum bilirubin, hemoglobin-hematocrit, reticulocyte count, and other individualized tests to determine the degree of red blood cell destruction and the need for blood transfusion or exchange transfusion. Severity of hemolytic disease and the normal hematologic values for each newborn infant were determined according to standard definitions.

Spectrophotometric analysis of amniotic fluid (delta optical density at 450 nm, ΔOD_{450}) was used as the main parameter in management of these pregnancies. There were no fetal or neonatal deaths.

For maternal IgG subclassing, we used antisera specific for each of the subclasses as antiglobulin reagents in the indirect Coombs test. This agglutination test was performed in glass capillary tubes. Antisera for IgG subclassing were obtained from the Netherlands Red Cross Transfusion Service (Amsterdam). A variation of the two-layer albumin capillary test of Crawford et al.[12] was used to determine IgG subclasses. Patients' sera were used to sensitize antigen-positive cells that were then washed five times and resuspended to a final concentration of 30% to 50% cells. A capillary tube was filled one third full with subclassing antisera; then an equal volume of 30% Pentex albumin was drawn into the tube followed by an equal volume of sensitized red blood cells. The tube was inverted and embedded in clay at a 60-degree angle and observed for agglutination with a ×7 hand lens as the cells fell to the bottom. A negative control consisting of 6% albumin was run with each different sensitized cell tested. Anti-IgG4 antiserum reacts with some IgG2 molecules; so, if IgG4 is detected, no definite conclusion about the presence or absence of IgG2 can be drawn.

Results

Table I summarizes the laboratory findings and pregnancy outcome in 22 patients studied. Nine patients had anti-D antibody; one of these also had anti-Js^b. The other 13 patients had irregular antibodies, one patient having both anti-Kell and anti-Jk^a. We detected 24 antibodies in the 22 patients. The subclass composition consisted of a single IgG subclass in eight antibodies and multiple IgG subclasses in 16 antibodies. All anti-D antibodies consisted of multiple subclasses. IgG1 and/or IgG3 was present in all cases.

Direct Coombs tests of cord blood samples were negative in five cases, and cord blood was not obtained from the baby of a Kell-sensitized mother whose husband was Kell negative (Case No. 20). One patient was delivered of her infant in another institution. These seven cases were excluded from further analysis. Direct

Coombs tests of cord blood were positive in 12 cases. Three other babies were severely affected and had received intrauterine transfusions; thus cord blood direct Coombs testing was not applicable to them. These 15 patients were considered susceptible to hemolytic disease of the newborn (susceptible group).

Of the 15 patients in the susceptible group, 11 were delivered of babies with hemolytic disease of the newborn (HDN group), and four were delivered of babies with no evidence of hemolytic disease of the newborn (no HDN group). Of the HDN group, seven babies were born following spontaneous labor and had mild hemolytic disease of the newborn (cord blood hematocrit of 34% to 66%, reticulocytosis above normal range for gestational age, and/or hyperbilirubinemia), one baby was delivered at 34 weeks' gestation and required transfusion of maternal blood for hemolytic anemia (moderate hemolytic disease of the newborn), and four babies had severe hemolytic disease of the newborn and required one or more intrauterine blood transfusions and/or postnatal exchange transfusions.

The susceptible group was divided into three subgroups: patients with IgG1 only, patients with IgG1 and IgG3, and patients with IgG3 only (Table II). Subclasses of IgG2 and IgG4 were not included in the analysis of pregnancy outcome. Five patients had IgG1 only; one was delivered of a moderately affected baby, and four had no evidence of hemolytic disease of the newborn. Eight patients had both IgG1 and IgG3, and all of the infants had hemolytic disease of the newborn. Two patients had IgG3 only, and both infants had hemolytic disease of the newborn (Table II). Comparison of pregnancies with IgG1 only and pregnancies with both IgG1 and IgG3 shows significantly increased rates of hemolytic disease of the newborn in the latter group. The number of pregnancies with IgG3 only is too small for a meaningful statistical analysis.

Table II. Correlation of maternal IgG subclasses with development of hemolysis in susceptible fetuses and neonates

	Hemolytic disease of newborn	
IgG subclass	*Present*	*Absent*
IgG1 only	1 (9)	4 (11,13,14,15)
IgG1 and IgG3	8 (1,2,3,4,5,6,8,10)	0*
IgG3 only	2 (7,12)	0

Numbers in parentheses represent case numbers.
*P = 0.007, Fisher's exact test.

Comment

The five major classes of antibodies in humans are IgG, IgM, IgA, IgD, and IgE. Blood group antibodies are usually varieties of IgG, IgM, or IgA.[13] Trophoblastic receptors bind only IgG, not IgM or IgA. Maternal IgG antibodies transported across the placenta can protect against infections or be harmful to the fetus. Passage of maternal IgG to the fetus in cases of maternal Graves' disease, myasthenia gravis, and immune thrombocytopenic purpura might cause transient manifestations of the disease in the newborn infant.

IgG antibodies are the most frequent type of red blood cell antibodies produced in isoimmunized patients.[5] Trophoblasts bind and engulf IgG molecules (pinocytosis), transport them across the cell, and release the antibody on the fetal side. Active transport of IgG across the trophoblastic barrier can result in higher antibody titers on the fetal side.[6] However, the presence in the maternal circulation of IgG antibodies against antigen-bearing fetal red blood cells is not invariably associated with fetal or neonatal hemolysis.[7] Furthermore, the severity of hemolysis when it does occur does not correlate well with antibody titers in maternal circulation.[7] The results of our study agree with these earlier findings.

IgG antibodies are divided into four subclasses: IgG1 to IgG4 with differences in biologic and immunologic properties. IgG molecules have an antigen-binding fragment (Fab) and a phagocytic cell–binding fragment, or crystallizable fragment (Fc). The Fc portion is constant for each subclass of IgG.[14]

Phagocytic cells have receptor sites for the Fc portion (FcR-IgG), and only IgG1 and IgG3 have Fc portions that can react effectively with phagocytic FcR-IgG. IgG2 and IgG4 are totally inactive in this regard.[5, 8, 10] Presence of IgG antibodies on red blood cells per se does not significantly affect red blood cell survival.[5] However, antibody-coated erythrocytes are destroyed when they come in contact with and adhere to monocytes-macrophages.[9]

Binding of red blood cell–antibody complex to receptor sites on effector cells is inhibited by the presence of free IgG molecules[5]; this phenomenon is useful in isoimmunization prophylaxis. Heavily sensitized erythrocytes are destroyed whenever they come in contact with macrophages. However, in most isoimmunized patients, sensitized red blood cell sequestration and destruction are confined to the spleen where special circumstances favor adherence of sensitized red blood cells to macrophages. Because of hemoconcentration in the spleen, the amount of IgG is relatively low, and stasis occurs.[5] So, on reaching the spleen, antibody-coated erythrocytes adhere to macrophages, and cytotoxic damage, spherocyte deformation, and eventual phagocytosis of the erythrocyte occur.[9]

IgG1 is produced in larger amounts and is transported across the placenta more efficiently and much earlier than other subclasses.[15] IgG3 has a much greater affinity for phagocytic Fc receptors than does IgG1,

and so the former is more efficient in binding sensitized red blood cells to macrophages.[5] Small (and sometimes undetectable) amounts of IgG3 can cause fetal and neonatal hemolysis, while much higher titers of IgG1 might not be associated with hemolytic disease of the newborn.[5] Schanfield[16] has shown that infants born to mothers with only IgG1 have a lower hemoglobin at birth, a situation suggesting a longer period of cell destruction in utero. Infants born to mothers with IgG3 have a higher cord hemoglobin, but the rate of bilirubin rise is faster after birth. Because of intrauterine and neonatal therapeutic interventions, we have been unable to evaluate these aspects of the actions of IgG subclasses. However, our results confirm the findings of Kline[14] that IgG1 is the most predominant subclass of IgG isoantibodies produced in isoimmunized pregnancies and IgG3 is the most efficient antibody in bringing about fetal and neonatal hemolysis.

IgG1 was detected in the majority of our cases and was associated with development of hemolytic disease of the newborn in nine of 13 (69.2%) susceptible cases. IgG3 was detected less frequently but was associated with hemolytic disease of the newborn in all susceptible babies. When IgG1 was detected in the absence of IgG3, one of five neonates developed hemolytic disease of the newborn. Two patients had IgG3 only; both neonates had hemolytic disease of the newborn. Unfortunately, the limited number of patients in this series does not allow conclusive comparisons of patients with IgG1 only and IgG3 only.

In summary, biologic behavior and immunologic properties differ among IgG subclasses, and determination of subclass IgG blood group antibodies might prove to be a useful adjunct in evaluating isoimmunized pregnancies. However, the information on correlation of IgG subclasses and fetal and neonatal hemolysis is limited and should not be used exclusive of traditional means of diagnosing and managing isoimmunized pregnancies.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. CHARLES M. STEDMAN, Birmingham, Alabama. Because of passage across the placenta, the IgG antibody molecule is the class of immunoglobulin of most interest to the obstetrician. Functionally, the Fc portion acts as the site of macrophage fixation and also permits active transport of the molecule by trophoblastic tissue. The Fab moiety contains the attachment sites for alloantibodies.

Once it was discovered that IgG could be divided by immunologic means into four subclasses, the hunt was on to correlate biologic activity with the type of subclass. Structurally, it is the heavy chain that provides the allotypic markers distinctive to each subclass. Taslimi and associates have chosen to address this controversial topic with a stated purpose of investigating 22 pregnancies complicated by isoimmunization. I commend the investigators on the laboratory technique selected and the use of negative controls with each specimen subjected to subclass characterization.

It is unfortunate that after reviewing the data in Table I, a total of eight patients must be removed from consideration either because the newborn infant was negative for the antigen or because a cord blood specimen was not collected. The remaining 14 patients may be separated into those with anti-D and those with irregular antibodies. Within the anti-D subset, one of nine patients demonstrated subclass 1 without 3, one patient manifested subgroup 3 without 1, and the remaining seven of nine showed a combination of subclasses 1 and 3. The irregular antibody subset contained subclass 1 without 3 in four of five patients.

My main concern with the study involves the analysis of results rather than the data collected. Table II is depicted as showing the correlation of maternal IgG subclass with the development of perinatal hemolysis.

A 2 by 2 table has been constructed when, in my opinion, there are three categories: IgG1 without IgG3, IgG3 without IgG1, and IgG1 with IgG3. When the data are rearranged, it is no longer possible to attribute any increased severity to the subclass 3 group.

The largest group in most previously reported studies has consisted of patients producing IgG1 combined with IgG3 antibodies. One inherent confounding aspect of this group is the inability to determine if there is predominantly IgG1 or IgG3 present. In a large study published earlier, Parinaud et al.[1] reported that severe hemolysis was most commonly associated with IgG1 in patients with anti-D. They even speculated that the subset of subclass 1 plus 3 might be protective when compared to subclass 1 alone. As a partial explanation for the conflicting findings in the literature, I submit that the quantity of erythrocyte-bound IgG may be as significant as the subclass identified. With the use of an enzyme-linked immunosorbent assay technique, preliminary data from our laboratory at the University of Alabama support this contention.

How does all of this translate into information useful for the clinician? As luck would have it, few erythrocyte alloantibodies are devoid of subclasses 1 or 3. As an example, Hunt et al.[2] have subclassified 86 antibodies of patients isoimmunized to 11 different antigens. Only four of 86 specimens tested failed to react with both IgG1 and IgG3. Therefore, except for the rare patient producing IgG antibody restricted to subclass 2 or 4, those of us managing patients with erythrocyte alloantibodies will continue to rely on antibody titers and more important amniotic fluid specimens for ΔOD_{450} values. Realistically, at this time, IgG subclassing remains a test looking for a clinical application.

In summary, I congratulate the authors for contributing to this area of interest, although I am unable to agree with their interpretation of the data. I look forward to a larger series from this group in the near future.

I have two questions for Dr. Taslimi. First, since the appearance of various subclasses can vary with the trimester, were the blood specimens analyzed for subclasses obtained just before delivery or several months earlier? Second, was the rate of neonatal bilirubin elevation any more rapid in newborn infants with only subclass 1 as opposed to those with subclass 1 plus 3?

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Dr. James R. Scott, Salt Lake City, Utah. A blood test that could replace more invasive measures such as amniocentesis to monitor the severity of fetal involvement would certainly simplify the clinical management of Rh-immunized patients. Since serial anti-D titers are not sufficiently reliable for this purpose, the authors have investigated whether IgG subclass determinations might provide more useful information. This is an important issue since previous reports disagree about the relationship of IgG subclasses to the severity of hemolytic disease in the infant.[1-4] There is general agreement that IgG1 tends to cross the placenta earlier in pregnancy than IgG3, but there are conflicting data regarding which has stronger hemolytic properties.[4-6]

Unfortunately, the present series contains patients with a variety of antibodies, and there are too few patients from whom to draw definite conclusions. The clinical outcome of the infant appears to correlate best with the specificity of the antibody rather than titer or subclass. Nevertheless, the authors state that "determination of subclass IgG blood group antibodies might prove to be a useful adjunct in evaluating and managing isoimmunized pregnancies." On the basis of their data, perhaps they could explain in more detail how they propose that this should be done. It seems to me that neither the antibody titer nor the IgG subclass has sufficient predictive value to manage an individual patient. For example, Patients 8 and 10 in Table I of the article had identical anti-D titers of 1:512 and both had only subclasses IgG1 and IgG3. However, the amniotic fluid ΔOD_{450} values from one mother were in zone II and her infant was only mildly affected, while the amniotic fluid ΔOD_{450} values from the other mother were in zone III and that infant had severe hemolytic disease requiring an intrauterine transfusion. Don't these cases illustrate that only with the conventional use of amniotic fluid ΔOD_{450} values (and perhaps ultrasound findings) can differences in fetal involvement be properly evaluated and appropriately managed? Perhaps the authors could comment on this.

Finally, an interesting new developement reported recently is the possibility that large doses of intravenous immune globulin may be able to compete with Fc receptor sites to prevent the placental transfer of endogenous maternal anti-D antibody.[7] Clearly, this would be a clinically useful maneuver and warrants further investigation. It has been shown that all the IgG subclasses in exogenously administered immune globulin cross the placenta,[8] and this also has important implications for the treatment of autoimmune diseases in pregnancy and prevention or treatment of fetal infections. Therefore it is likely that the last word on antierythrocyte antibody subclasses, their relationship to hemolytic disease of the newborn, and potential ways to manipulate or prevent placental passage is not yet in. I commend the authors for renewing interest in this area and encourage them to pursue their investigations along these lines.

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Dr. Sibai (Closing). I would like to thank both reviewers regarding their relevant and useful comments. I agree with them regarding the small number of patients studied in this report. However, as of September, 1985, we had studied 20 additional patients with IgG subclasses, for a total of 42 patients. Twenty-four of the 42 were considered susceptible to development of hemolytic disease of the newborn. In these 24 patients, 16 had babies with hemolytic disease of the newborn, while eight neonates did not develop hemolytic disease of the newborn even though they had a positive direct Coombs test. The IgG3 subclass was detected in 12 of 24 susceptible cases and hemolysis developed in all 12 babies. However, the IgG1 subclass was detected in 22 of 24 susceptible cases, but hemolysis developed in only 14 babies. Table I summarizes and compares neonatal outcome with the presence of either IgG3 or IgG1. There was a significant difference ($p < 0.005$) regarding the development of hemolytic disease of the newborn between cases with IgG3 present and those without IgG3.

Dr. Stedman asked about the time of determination of IgG subclasses during pregnancy. The samples were obtained during the third trimester before delivery. Serial determinations of IgG subclasses were not obtained except in two patients. However, such determinations were reported by Frankowska and Gorska[1] as well as by Parinaud et al.[2] These authors found that the pattern and titer of subclasses may change during pregnancy. In regard to his second question concerning the relationship between the IgG subclasses and the rise in neonatal bilirubin levels, the findings suggest that increased neonatal bilirubin levels are associated with the IgG3 subclass while higher cord bilirubin levels are usually associated with IgG1.[2,3]

Table I. Correlation between IgG subclasses and neonatal outcome

	*IgG3 subclass**		*IgG1 subclass†*	
	HDN	*No HDN*	*HDN*	*No HDN*
Present	12	0	14	0
Not detected	4	8	2	0

HDN = Hemolytic disease of the newborn.
*$p < 0.001$.
†NS.

Dr. Scott questioned the potential value of IgG subclasses in the management of isoimmunization considering the variable neonatal outcome with similar IgG subclass titers. He emphasized the importance of amniocentesis as the only reliable clinical method for follow-up of such pregnancies. I fully agree with his remarks. I believe that IgG subclass determinations should be considered for research purposes at present. However, they might be potentially useful in rare cases, such as in patients with isoimmunization and sickle cell disease with hepatopathy or hepatitis wherein amniotic fluid values may reflect the elevated maternal bilirubin levels.

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Experience with intrauterine insemination for cervical factor and oligospermia

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In the past 100 years, interest in intrauterine insemination for cervical factor has waxed and waned. Recently the advent of in vitro fertilization, with improved methods of sperm washing and preparation, has revived interest in intrauterine insemination as a modality for the treatment of infertility. Twenty-seven couples with infertility resulting from cervical factor and/or oligospermia were entered into our intrauterine insemination protocol. A total of 107 intrauterine inseminations were performed in 85 menstrual cycles. Nineteen couples with a cervical factor as the major factor in their infertility experienced three pregnancies (16%). The remaining eight couples who had oligospermia with or without cervical factor produced no pregnancies. No major complications occurred from this procedure. In view of the low pregnancy rate experienced in this study, we must seriously question the value of intrauterine insemination in the treatment of cervical factors and/or oligospermia. (AM J OBSTET GYNECOL 1986;154:1333-8.)

Key words: Cervical factor, intrauterine insemination, oligospermia

Intrauterine insemination has been a subject of controversy for the past 100 years.[1] Recently with the development of in vitro fertilization and accompanying methods of sperm washing, intrauterine insemination is being increasingly used to treat infertility almost irrespective of its apparent cause. Thus indications for intrauterine insemination have been extremely varied, vague, inconsistently applied, and oftentimes empiric. Reported pregnancy rates have varied from as low as 3% to as high as 75%. For these reasons this study was performed. Although it is beyond the scope of this paper to consider all possible indications for intrauterine insemination, it is our purpose in this study to evaluate the merits of intrauterine insemination for the treatment of couples with infertility resulting from cervical factors, with or without accompanying oligospermia.

Material and methods

Patient selection. Couples with other infertility factors that were untreated were eliminated from the study. A careful history was obtained and physical examinations performed for all couples. Infertility evaluation for all couples included a hysterosalpingogram, multiple semen analyses, basal body temperature charting, and luteal phase endometrial biopsies. Laparoscopy and tubal dye perfusion were performed when indicated.

Cervical infertility was diagnosed initially by the performance of at least two well-timed postcoital tests. Cervical mucus was scored according to the scheme recommended by the World Health Organization.[2] Additional tests performed to confirm abnormalities of sperm-cervical mucus interaction included hyperstimulation with estrogen, in vitro sperm-cervical mucus penetration tests, cross-hostility tests, and insemination testing. The presence of sperm antibodies were excluded by performing sperm immobilization and sperm-agglutinating antibody testing in sera, cervical mucus, and seminal fluid of the couple.

Oligospermia was diagnosed after two semen analyses, performed at least 2 weeks apart, were evaluated according to the criteria recommended by the World Health Organization.[3] Those men who had a total sperm count below 120 million or less than 20 million sperm per milliliter of seminal fluid were considered oligospermic.

Sperm preparation. Couples were asked to abstain from intercourse for at least 48 hours before specimen collection. Semen samples were obtained by masturbation and allowed to liquefy for 30 minutes. Sperm were counted on a Neubauer ruled hemocytometer. Motility and morphology were determined according to the guidelines set by the World Health Organization Standard for Rating Sperm Morphology.[3]

Ham's F10 media was prepared in advance and adjusted for pH and osmolarity. Sera from the wife was prepared in advance in 1 ml aliquots, detoxified at 56° C for 30 minutes, and stored at −70° C until in-

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Table I. Comparison of results by indication

	Cervical factor	*Male factor (oligospermia)*	*Male factor and cervical factor*	*Total*
Patients	19	4	4	27
Cycles	65	11	9	85
Insemination	81	14	12	107
Pregnancy (n and %)	3 (16)	0 (0)	0 (0)	3 (11)

Table II. Overall comparison between ejaculates and recovered sperm in prewash and postwash specimens

Specimen	*Volume (cc)** (mean ± SD)	*Count/millions/ml†* (mean ± SD)	*Percent of motility* (mean ± SD)	*Normal morphology†*
Prewash	2.86 ± 1.35	85.21 ± 69.6	50.7 ± 5.85‡	58.86
Postwash	0.81 ± 0.25	66.47 ± 66.6	48.79 ± 7.58‡	60.58

*$p < 0.001$.
†$p < 0.05$.
‡NS.

semination. On the day of insemination the 1 ml aliquots were thawed to 37° C and a 7.5% solution containing the wife's serum and Ham's F10 media was prepared before sperm washing.

Semen samples were admixed in equal volumes with the 7.5% serum and Ham's F10 solution and centrifuged at 427 g for 7 minutes. The supernatant was discarded, and the pellet was resuspended with 2 ml of media and recentrifuged at 427 g for 7 minutes. The remaining pellet was then resuspended in 1 ml of media and incubated for 30 minutes at 37° C in 5% CO_2. During the incubation period the nonmotile sperm are allowed to settle out while the motile sperm remain in the supernatant. The supernatant was then removed and reassessed for sperm count, motility, and morphology.

Insemination, timing, and technique. Couples were inseminated at the time of ovulation as determined by basal body temperature and rapid urinary LH assay (Ovustick, Monoclonal Antibodies, Inc., California) performed on a daily basis beginning on cycle day 10.

Intrauterne insemination was performed under sterile conditions with a tuberculin syringe filled with sperm suspension attached to an 18-gauge angiocatheter 2 inches in length.

Statistical analysis. Means and standard errors of the means as well as standard deviations were calculated. Differences between normal and oligospermic men were evaluated by paired *t* test and correlated by computer analysis.

Results

Twenty-seven couples either with primary or secondary infertility underwent intrauterine insemination. The mean (±SD) age of the women who participated was 31.96 ± 0.80 years. The duration of infertility was 4.64 ± 0.72 years. Five of the women had previous conization or cryotherapy of the cervix for dysplasia, and one had a history of exposure to diethylstilbestrol in utero. Eight men were thought to have idiopathic oligospermia unresponsive to usual therapeutic modalities.

A total of 107 inseminations were performed in 85 cycles with a total of 3.12 cycles per couple as seen in Table I. Of the twenty-seven couples inseminated, 19 patients had only a cervical factor. No pregnancies occurred in the four couples with oligospermia or in the four couples with oligospermia as well as cervical factor.

Occasional minor complications were noted such as spotting in three cycles and mild discomfort after insemination in two patients. No major complications were observed.

Overall, as shown in Table II, there is a statistically significant difference, as expected, in ejaculate volume before and after washing ($p < 0.00$_). The observed sperm count is significantly different in the prewash and postwash specimens, in that the mean sperm count fell from 85.21 million per milliliter to 66.47 million per milliliter ($p < 0.05$) following the washing procedure. In addition, sperm motility was observed to be less after washing—48.79% as compared to a prewash value of 50.7%—but was not statistically significant. The percentage of sperm demonstrating normal morphology was found to increase from 58.65% to 60.58% ($p < 0.05$) in the postwash specimen.

Differences were noted in the prewash and postwash specimens of normal versus oligospermic men. As shown in Table III, there were differences in volume between raw ejaculates and washed sperm regardless of the sperm counts before washing. Subjecting sperm

Table III. Comparison between ejaculates and recovered sperm in two defined categories: normal and oligospermic

	Normal (n = 19)		*Oligospermic (n = 8)*	
	Ejaculate (mean ± SD)	*Recovered sperm (mean ± SD)*	*Ejaculate (mean ± SD)*	*Recovered sperm (mean ± SD)*
Volume	3.24 ± 1.29*	0.83 ± 0.28*	2.11 ± 1.10*	0.76 ± 0.16*
Sperm count (millions/ml)	108.34 ± 74.40§	88.71 ± 70.52§	42.08 ± 27.10‡	21.51 ± 13.77‡
Motility (%)	51.09 ± 6.24§	49.86 ± 7.81§	49.73 ± 4.85§	46.49 ± 6.55†
Normal morphology (%)	60.33 ± 15.06§	64.69 ± 18.87§	72.50 ± 8.00§	54.29 ± 15.34§

*$p < 0.05$.
†$p < 0.1$.
‡$p < 0.001$.
§NS.

from normal men to washing does not seem to change the recovered amount of sperm significantly. However, those men with oligospermia had significantly fewer sperm recovered after sperm washing compared to their prewash counts. Also the overall mean percentage of motile sperm changed significantly in the postwash specimens of the oligospermic men from 49.7% to 46.5%. The mean percentage of sperm demonstrating normal morphology increased postwash in normal men and decreased postwash in oligospermic men, but neither change was statistically significant.

We also examined total sperm counts (multiplying volume by count per milliliter) in both normal and oligospermic men. As expected, the total count decreased after sperm washing. Other aspects such as motility and morphology when evaluated (by multiplying percent of motility and of morphology by total count) were found to decrease.

Comment

In the past, intrauterine insemination has often been described as disadvantageous and even dangerous.[1] Intrauterine insemination was initially performed with use of the entire ejaculate, and comptemporary reports listed complications such as anaphylaxis and pelvic infection. It was later recognized that the seminal plasma was a possible vehicle for infection, and the procedure was abandoned. The discovery of prostaglandins in seminal plasma along with the popularization of split ejaculates for artifical insemination by the husband led to a revival of intrauterine insemination.[1-6] The use of small volumes and split ejaculates has lessened the incidence of pain and complications associated with intrauterine insemination.[7-9] The use of sperm washing and aseptic techniques, in our hands, reduced the complications to mild uterine cramping and occasional vaginal spotting.

The results of our study, however, do not show any significant improvement in the rate of pregnancy by use of washed sperm and intrauterine insemination techniques. In fact, our results are no better than that obtained in our previous reported experience with intracervical insemination techniques and unwashed husband sperm.[10] We believe that this is due to the failure of washing techniques to improve sperm quality in any marked way. Instead of improving the quality of inseminated sperm, our technique appeared to actually decrease the number of sperm inseminated without any improvement in motility as has been substantiated by others.[11]

When the prewash and postwash specimens were compared in relation to normal and oligospermic men, differences became apparent. Postwash sperm counts and motility actually decreased when compared to prewash specimens in the oligospermic group. This is in contrast to data reported earlier by others.[12] However, the sperm count and motility of the group of normal men did not change significantly. Therefore, we believe it is the overall quality of sperm before sperm washing and not the technique of sperm washing that should be held accountable for the lack of improvement in quality of postwash specimens.

In this study we were very careful to limit the performance of intrauterine insemination to couples in whom there actually existed an indication for this procedure. The use of intrauterine insemination and sperm washing techniques in this group of patients with infertility resulting from cervical factors and/or oligospermia did not result in an increased rate of pregnancy over other conventional methods of therapy.[10] We believe that this is the probable result of the inability of our sperm washing technique to functionally improve the quality of postwash sperm either in normal men or especially in oligospermic men. Results obtained by Confino et al.,[13] in a recent study using intrauterine insemination techniques, were similar. Their overall pregnancy rate for all couples was 30%, but none of the couples with oligospermic men achieved pregnancy. Their most encouraging results came in the group of patients with hostile cervical mucus in which the preg-

nancy rate was 68%.[13] Similarly, couples with cervical factors conceived at a rate of 75% per patient following intrauterine insemination, while couples, overall, demonstrated a pregnancy rate of 28% per patient in a recent report by Kirsch et al.[14]

Thus it would seem that intrauterine insemination may yield extremely high rates of pregnancy in couples with cervical factors including hostile cervical mucus. Although the pregnancy rate in couples with cervical factor only in the present study was merely 16%, all couples had previously exhausted other more conventional treatment for cervical factors before proceeding to intrauterine insemination. It is not clear at this time whether couples in other studies were similarly treated before undergoing intrauterine insemination.

Intrauterine insemination with use of sperm washing techniques requires a sophisticated laboratory with personnel available on a daily basis. Sperm preparation is time consuming and more expensive than conventional insemination techniques with use of intracervical or intravaginal sperm insemination. Until further, more extensive and controlled studies can be performed, we are concerned that intrauterine insemination may offer no added benefit for the treatment of infertility related to oligospermia. Further study may more clearly define its role in the treatment of cervical factors that are not responsive initially to more conventional methods of treatment.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. DENNIS J. LUTZ, Minot, North Dakota. Intrauterine insemination remains a subject of controversy despite a quarter-century of increasing efforts to evaluate the merits of the technique. Inconsistent and occasionally contradictory reports on the successful treatment of infertile couples with intrauterine insemination have not adequately resolved this controversy. The authors have selected a relatively small subset of couples with infertility caused by cervical factors, with or without accompanying oligospermia, and attempted to assess the merits of intrauterine insemination by means of washed homologous semen. Several aspects of the study design require clarification.

The selection of couples for this study required cervical factor infertility based on the diagnosis of repeated "poor" postcoital test results, implying that infertility was due to abnormal sperm-mucous penetration.[1] Nevertheless, the average age of each woman was 32 years and the average duration of infertility approximately 5 years. This would suggest a study bias toward older couples who may have other unexplained infertility factors in addition to the cervical and/or oligospermia factors. A more detailed fertility profile of these couples, including primary and secondary infertility data, might be elucidating. No mention is made of hormonal therapy for cervical factor infertility in this same study cohort, either before, in conjunction with, or after attempted intrauterine insemination cycles.

Furthermore, only an average of three cycles of intrauterine insemination per couple were performed. Is that an adequate trial of the technique for this particular subset of extremely refractory infertility patients? In an earlier report for homologous artificial insemination by means of the split ejaculate technique, Moghissi et al.[2] required a prescribed course of six or more attempts.

Obviously the study cohort is small, particularly for these couples with oligospermia, with or without cervical factor; therefore, results for these two groups cannot be interpreted on the basis of intrauterine insemination techniques alone. Not only would a much larger study group be required, but also a control group using intrauterine insemination with washed donor semen would be useful. In any event, the use of washed semen and intrauterine insemination technique does not seem to improve pregnancy results for any group now or previously studied by the authors. Indeed, Glass and Ericsson[9] have concluded that even the best sperm from

a poor specimen may have intrinsic defects that interfere with their ability to fertilize.[3]

This report may dampen the enthusiasm of proponents of intrauterine insemination as a technique for bypassing cervical factor infertility and of sperm washing as a panacea for circumventing oligospermia. Our own data using unwashed, split ejaculate intrauterine insemination for cervical factor infertility is no more encouraging. Indeed the three pregnancies (16%) among the 19 couples diagnosed as pure cervical factor may represent either dismal or truly excellent results within this refractory infertile cohort. Unfortunately, that judgment cannot be determined from the data presented today.

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DR. ASGHAR AFSARI, West Bloomfield, Michigan. Artificial insemination was introduced in the clinical field, as a means to promote conception, by John Hunter in 1790.[1] Approximately 200 years later, it still remains a viable tool for infertility treatment. However, in some instances the value of this procedure has also been a myth. Widespread use of this procedure, with or without good clinical indication and technique, as well as imprecise timing and inadequate frequency of the procedure per cycle, verifies this.

This paper addresses itself to the treatment of couples with sperm and/or cervical infertility with the use of washed sperm and intrauterine insemination. I would like to comment on these two distinct areas of interest, as well as patient selection and methodology.

First, while oligozoospermia, asthenozoospermia, or combination of both continues to be by far the most frequent abnormalities encountered, varicocele is considered to be the most frequent etiologic factor. I am interested in learning whether safeguards were taken by the authors to rule out this condition. Although it is simple to detect a varicocele, occasionally the detection of an occult varicocele is quite enigmatic.

So far as the treatment for sperm factor is concerned, newer and more contemporary means are advised. Administration of gonadotropins to subfertile men is reported to have achieved a 40% improvement.[2]

It has been shown[3] that intrauterine insemination leads to a 25% increase in peritoneal recovery of the sperm, when compared to intracervical insemination. Now the ever-popular form of intrauterine insemination, with the use of capacitated sperm, for the purpose of selection of more motile sperm, has been shown to be a superior method. The technique of sperm washing as described by the authors of this paper, shows that the quality of the sperm not only was improved but surprisingly had a deleterious effect, resulting in an overall pregnancy rate of only 11%. This is contrary to the work of Balmaceda et al.,[4] who achieved an overall improvement of sperm motility from 51.7% to 85% and obtained a 50% pregnancy rate. It is also interesting to note that, nevertheless, a 14% pregnancy rate is expected to occur spontaneously after discontinuation of a regimen of homologous insemination in a group of subfertile men.[5]

Second, various courses of treatment are advocated when cervical infertility is encountered. These include low-dose estrogen stimulation, antibiotic therapy, cryotherapy of the cervix, donor cervical mucus, and last, intrauterine insemination. An encouraging result of 68% to 75% pregnancy rates is at hand with the use of washed sperm for intrauterine insemination.

Dr. Hull and her colleagues denote that all couples had previously exhausted other conventional treatment for cervical infertility before proceeding with intrauterine insemination. I wonder if administration of human menopausal gonadotropin plus human chorionic gonadotropin with or without daily high doses of estrogen also had been tried. The above therapy is reported to render a better success rate in achieving pregnancy.[6, 7] Bearing in mind that artificial insemination causes an anxiety reaction in couples, resulting in temporary oligoanovulation, this method of treatment for the most part may override the wife's emotional upheaval, caused by their infertility problems in general, but more specifically by the need for artificial insemination.

Third, the methodology designed by the authors of this paper deserves more comment. They have acknowledged that laparoscopy was performed when it was indicated. Does this mean that it was not performed on all patients? If this is the case, I believe that the extent of couple evaluation to rule out any pelvic pathologic conditions was incomplete. In comparison, Kirsch et al. performed laparoscopy on all patients before the intrauterine insemination. They were then able to demonstrate the pregnancy rate of 75% in couples with cervical infertility and only 5% in the group with history of endometriosis. This phenomenon is more than likely due to an altered prostaglandin secretion, an inherent type of problem with endometriosis. The low pregnancy rate of 16%, reported through this paper, may then be attributed to the poor selection of patients and also to failure to proceed with screening laparoscopy on all patients who entered the protocol. I wonder whether any of the patients who failed to conceive were subsequently given a diagnostic laparoscopy, and whether any organic pelvic pathologic condition or endometriosis was discovered.

In addition to above comments, I agree with the authors that their method of sperm washing is insufficient to produce a better quality sperm. My concern involves the use of the wife's serum as part of the washing medium and the use of supernatant alone, after the second wash, for the final specimen for intrauterine insemination. By modifying the two above areas, the authors

may find a higher rate of pregnancy as well as a simpler, faster, and consequently more gratifying technique.

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Dr. James Miller, Seattle, Washington. You stated that you "used laparoscopy when indicated." I would like to know when laparoscopy is not indicated in an infertility workup.

With regard to your timing, basal body temperatures are notoriously inaccurate, and the luteinizing hormone kits are not all that good either. Recently in Seattle we compared luteinizing hormone kits to serum luteinizing hormone determinations and found they were accurate only 75% of the time.

I agree with the discussants that in 30-year-olds three tries are hardly adequate to draw a conclusion.

Dr. Karol J. Chacho, Chicago, Illinois. In light of the normal attrition in the number of sperm that successfully migrate from the cervix into the uterine cavity, I wonder why you did not select a technique that would have decreased the concentration but perhaps increased the percent motility of your specimen.

Also, what is your feeling about depositing that concentration of sperm into the uterine cavity, and did you look at sperm antibodies after the procedures compared to before the procedures to see where there was any difference?

Dr. Hull (Closing). The diagnosis of cervical factor was indeed made after two well-timed postcoital tests and after poor results were obtained through in vitro sperm-cervical mucus penetration tests and cross-hostility tests, and there was failure to respond to high doses of ethinyl estradiol. The oldest patient we encountered was about 41 years old. The average age was 31.9 ± 4.2 years.

Cervical factor was treated hormonally before institution of insemination therapy, that is, with high-dose estrogens, either Premarin (1.25 mg) or ethinyl estradiol (20 mg) daily for a total of 14 days, and then withdrawal with Provera. If they had responded with production of cervical mucus, they were not included in this study. If they had not, they then proceeded on to intrauterine insemination.

Regarding the question of whether three or more cycles is adequate, I do agree that this is not. We are continuing to study this further, and thus this is a preliminary report of our data.

Dr. Afsari raised the question about the use of estrogen therapy in conjunction with Pergonal and human chorionic gonadotropin. We found that if the patients were not responsive to high doses of ethinyl estradiol or Premarin, we then proceeded to do sperm washing along with intrauterine insemination because we felt that they were more unlikely to respond to Pergonal (human menopausal gonadotropin). Indeed, some patients in the group did go on to Pergonal therapy and did not produce mucus during those cycles and also required intrauterine insemination.

A question was raised whether or not all patients had laparoscopy to rule out other factors such as endometriosis. All patients did not have laparoscopy, and I agree that this may be part of the fault of the study; but they did demonstrate patency of the tubes. Twenty patients had laparoscopy before intrauterine insemination, and 18 were not found to have endometriosis. Two had minimal disease. The remaining seven patients did not have a laparoscopy.

Dr. Miller raised the question that the luteinizing hormone kits were unreliable in his experience. We have not found any problems with luteinizing hormone kits. We have used them in conjunction with luteinizing hormone assays in our in vitro fertilization program and have not found a problem with them.

Concerning the question about postwash formation of antibodies, the reason we used a 7.5% solution of the wife's sera was that we wished not to stimulate any antibody formation, and we used the woman's own proteins. I do know of one study that used intrauterine insemination in patients with a history of antisperm antibodies, and the titers were elevated significantly after several inseminations. However, in cases known to have cervical factor without antisperm antibodies, the answer is unknown.

An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears

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Two thousand one hundred ninety-four patients with abnormal Papanicolaou smears were evaluated by colposcopy without endocervical curettage in the initial part of the series but with routine endocervical curettage in the latter part. Forty-two cases of microinvasion-invasion were all accurately diagnosed with no postcryotherapy occurrence of these lesions. Evaluation of the Papanicolaou smear, colposcopic biopsy specimens, squamocolumnar junction location, and use of surgical removal for severe dysplasia–carcinoma in situ appeared to be most important in the successful outcome. Endocervical curettage played a minor role. Multiple positive cross correlations between Papanicolaou smear severity, colposcopic biopsy specimen severity, squamocolumnar junction nonvisualization, positive results of endocervical curettage, increased lesion size, increased age, and microinvasion-invasion occurrence were all demonstrated.
(AM J OBSTET GYNECOL 1986;154:1339-49.)

Key words: Colposcopy, Papanicolaou smears, cervical dysplasia

This report attempts to analyze the contribution of several variables to the successful use of colposcopic triage as performed at the colposcopy clinic at University Hospitals from its inception in 1971 through 1983. The variables analyzed include the degree of Papanicolaou smear abnormality, histologic results of colposcopic biopsy, squamocolumnar junction location, endocervical curettage, lesion size, and patient age.

Material and methods

A total of 2194 patients with abnormal Papanicolaou smears were evaluated by colposcopy by means of standard techniques in a resident clinic supervised by myself. From 1971 to 1976, endocervical curettage was rarely used, wereas it was almost always used from 1977 to 1983.

Results

Identification of patients with microinvasion or invasion was a major objective with 42 patients having one of these diagnoses. Before 1977, there were eight patients with invasive carcinoma and 18 with microinvasion, and after 1977, there were 11 patients with invasive carcinoma and five patients with microinvasion for a total of 19 patients with invasive carcinoma and 23 patients with microinvasion. In all instances these patients were identified as a result of the initial evaluation steps, and no diagnoses were delayed by inappropriate cryotherapy or advice to have a repeat Papanicolaou smear or colposcopy after a waiting period. No patients developed these lesions after cryotherapy. The successful accomplishment of the colposcopic evaluation was dependent on the analysis of a number of variables, which included Papanicolaou smear severity, pathologic results of colposcopic biopsy, squamocolumnar junction visualization, endocervical curettage findings, lesion size, and patient age. The data from our series regarding these variables will be reviewed.

The degree of Papanicolaou smear abnormality is correlated with the severity of the subsequent colposcopic biopsy results. This is displayed in Table I. It can be noted that there was 85.9% agreement between Papanicolaou smear and colposcopic biopsy results. A total of 4.5% had a Papanicolaou smear result much more severe than the colposcopic biopsy result and 9.6% had a colposcopic biopsy result much more severe than the Papanicolaou smear. The 9.6% discrepancy indicates the false negative problem of Papanicolaou smears. As all the patients had abnormal Papanicolaou smears before referral it is of interest that 27% had normal Papanicolaou smears at the colposcopy clinic. Of those with normal Papanicolaou smears at colposcopy 43% had some degree of dysplasia on colposcopic biopsy. This demonstrates that after an abnormal Papanicolaou smear and with no intervening therapy a normal Papanicolaou smear has a high liklihood of being false negative. Comparing the colposcopic biopsy diagnosis with the colposcopic Papanicolaou smear revealed that 16.3% of all patients with dysplasia–carcinoma in situ of any degree on colposcopic biopsy had normal colposcopic Papanicolaou smears. Of those with mild dys-

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Table I. Papanicolaou smear versus colposcopic biopsy specimen

Papanicolaou smear at colposcopy	*Colposcopic biopsy specimen*				
	Normal	*Mild dysplasia*	*Moderate dysplasia*	*Severe dysplasia*	*Carcinoma in situ*
Normal	289	137	57	18	9
Mild dysplasia	94	182	109	43	14
Moderate dysplasia	35	99	163	70	22
Severe dysplasia	12	14	38	64	36
Carcinoma in situ	2	2	12	22	64

Table II. Association of microinvasion and invasion with various gradations of abnormality on Papanicolaou smear or colposcopic biopsy specimen (2194 patients, 1971 to 1983)

	No. of smears of specimens	*Microinvasion*	*Invasion*	*Chance of microinvasion or invasion (%)*
Papanicolaou smear at colposcopy	611	0	0	0
Normal				
Mild dysplasia	495	2	0	0.4
Moderate dysplasia	419	2	1	0.72
Severe dysplasia	185	6	1	3.8
Carcinoma in situ	136	6	5	8.1
Microinvasion	18	2	3	28
Invasion	20	4	9	65
Colposcopic biopsy specimen				
Normal	538	0	0	0
Mild dysplasia	538	2	0	0.37
Moderate dysplasia	478	0	1	0.21
Severe dysplasia	291	2	0	0.69
Carcinoma in situ	210	8	2	4.8
Microinvasion	11	10	1	100
Invasion	14	–	14	100
Entire series	2194	23	19	1.9

plasia on biopsy, 31.6% had negative Papanicolaou smears; of those with moderate dysplasia on biopsy, 15.0% had negative Papanicolaou smears; of those with severe dysplasia on biopsy, 8.3% had negative Papanicolaou smears; and of those with carcinoma in situ on biopsy, 6.2% had negative Papanicolaou smears. These figures demonstrate the false negative problem of Papanicolaou smears and also that the more advanced lesions are less likely to result in negative Papanicolaou smears.

Of interest also is the outcome of patients with a Papanicolaou smear that was significantly more severe than the colposcopic biopsy result. There were 42 patients with severe dysplasia or worse on Papanicolaou smear but with colposcopic biopsy results that were much less severe (greater than one grade). Thirty-three of these patients had subsequent cone biopsy and/or hysterectomy and the subsequent pathologic results were normal in five, mild dysplasia in five, moderate dysplasia in five, severe dysplasia in 11, carcinoma in situ in five, microinvasion in one, and invasion in one. These data demonstrate that the colposcopic biopsy also has a false negative problem that may be revealed by the Papanicolaou smear being significantly worse. Conversely the Papanicolaou smear has only a minor false positive problem.

The degree of Papanicolaou smear abnormality is also correlated with the likelihood of coexisting microinvasion or invasion and this is demonstrated in Table II. Even milder Papanicolaou smear abnormalities may be associated with advanced disease but with increasing Papanicolaou smear severity there is a strong correlation with the likelihood of microinvasion-invasion.

Colposcopic biopsy pathologic results are correlated with the ultimate pathologic diagnosis on cone biopsy or hysterectomy as displayed in Table III. There are discrepancies where the colposcopic biopsy underestimates the severity of the lesion and these discrepancies are most likely to occur when the squamocolumnar junction is not visualized or the endocervical curettage is positive. Significant discrepancies were found in 8.8%

Table III. Colposcopic biopsy pathologic results versus worst pathologic result on cone biopsy or hysterectomy in 500 patients, 1971 to 1983

Colposcopic biopsy pathologic result	*Cone or hysterectomy pathologic result*						
	Normal	*Mild dysplasia*	*Moderate dysplasia*	*Severe dysplasia*	*Carcinoma in situ*	*Microinvasion*	*Invasion*
Normal	14	5	4	6	1	–	–
Mild dysplasia	7	14	10	8	2	2	–
Moderate dysplasia	6	24	29	19	8	–	–
Severe dysplasia	17	26	36	60	30	2	–
Carcinoma in situ	8	8	14	22	89	8	2
Microinvasion	1	1	–	–	7	4	2
Invasion	–	–	–	–	–	–	4

with colposcopic biopsy results less severe by two gradations than subsequent pathologic findings in the 1971 to 1983 series of 500 patients who underwent cone biopsy and/or hysterectomy. Of these 44 patients 30 or 68% had nonvisualization of the squamocolumnar junction. In the 1977 to 1983 series where endocervical curettage was done, 17 of 264 patients had more severe abnormality at conization or hysterectomy than at colposcopic biopsy and 10 of 17 or 59% had a positive result of endocervical curettage. Cross correlation of squamocolumnar junction evaluation and endocervical curettage results in these discrepant cases from 1977 to 1983 is displayed in Table IV. In 15 of 17 discrepant cases either the squamocolumnar junction was not visualized or the endocervical curettage result was positive. The other clue to colposcopic biopsy discrepancies is the occurrence of a Papanicolaou smear with much worse findings, as mentioned earlier.

Visualization of the squamocolumnar junction is one of the two methods used to identify those patients with endocervical disease. The percentage of patients with visualization of the squamocolumnar junction was 80.3% before 1977 and 74.4% after 1977 or 77% for the entire series. Review of colposcopic biopsy diagnosis versus squamocolumnar junction visualization reveals that when the squamocolumnar junction was seen 9% of patients had carcinoma in situ, microinvasion, or invasion. When the squamocolumnar junction was not visualized, 21% had these more severe lesions. Of 219 patients with these more severe lesions, 41% had nonvisualization of the squamocolumnar junction and in 49% the squamocolumnar junction was seen. Visualization of the squamocolumnar junction in those patients with microinvasion and invasion is displayed in Tables IV, V, and VI. The data reveal that the squamocolumnar junction is more often not visualized in the more severe lesions and is particularly unlikely to be seen in patients with invasion.

Endocervical curettage is the other index of the presence of endocervical disease. This procedure was rarely used in our series before 1977 but since has been used routinely. In the 957 patients since 1977 who had an endocervical curettage, the endocervical curettage result showed moderate dysplasia or worse in 8.6%, mild dysplasia in 3.7%, and dysplasia unspecified in 1.7%. These 14% of patients are subsequently referred to as having positive endocervical curettage results. A total of 14.4% had insufficient tissue and 70% had benign tissue; these 84% of patients are subsequently referred to as having negative endocervical curettage results. Comparing the endocervical curettage result to the severity of the abnormality on colposcopic biopsy reveals that when the result was positive 24% of the patients had carcinoma in situ, microinvasion, or invasion and when the result was negative 6% of patients had these more severe lesions. Of the 79 patients who had these more severe lesions on colposcopic biopsy and had an endocervical curettage, 39% had a positive result and 61% had a negative result. The correlations of endocervical curettage results with microinvasion-invasion are displayed in Tables IV, V, and VI. It is obvious that the more severe lesions have a higher incidence of positive endocervical curettage results but also of note is that of 18 patients with microinvasion or invasion who underwent endocervical curettage five had a negative result. The endocervical curettage in addition to detecting endocervical disease may also yield tissue that may give a histologic diagnosis that is more severe than that found on colposcopic biopsy. In the total 1977 to 1983 series there were 35 patients with mild dysplasia on endocervical curettage and 88 patients with moderate dysplasia or worse. Of the 88 patients with mod-

Table IV. Direct comparisons of patients with both squamocolumnar junction visualization evaluation and endocervical curettage, 1977 to 1983

	Endocervical curettage result	
	Negative	*Positive*
Entire series		
Squamocolumnar junction		
Seen (77%)	649	50
Not seen (23%)	159	83
	(85%)	(15%)
Patients with carcinoma in situ		
Squamocolumnar junction		
Seen (47%)	36	7
Not seen (53%)	19	29
	(60%)	(40%)
Patients with microinvasion		
Squamocolumnar junction		
Seen (0%)	0	0
Not seen (100%)	2	2
	(50%)	(50%)
Patients with invasion		
Squamocolumnar junction		
Seen (0%)	0	0
Not seen (100%)	3	7
	(30%)	(70%)
Patients with colposcopic biopsy pathologic result much less severe than cone-hyst pathologic result		
Squamocolumnar junction		
Seen (24%)	2	2
Not seen (76%)	5	8
	(41%)	(59%)

erate dysplasia or worse on endocervical curettage, 22 were patients with the squamocolumnar junction seen. Of these 22 patients the endocervical curettage diagnosis was worse than the Papanicolaou smear or colposcopic biopsy diagnosis in four of 22 and much worse (greater than one grade) in two of 22. Six patients with a final diagnosis of microinvasion or invasion had the squamocolumnar junction seen and none had an endocervical curettage done. Among the 66 patients with moderate dysplasia or worse on endocervical curettage with the squamocolumnar junction not seen, the endocervical curettage result was worse than the Papanicolaou smear or colposcopic biopsy result in seven of 66 and much worse (greater than one grade) in two of 66. Thirty patients with microinvasion or invasion had the squamocolumnar junction not seen; an endocervical curettage was done in 17 and results were positive in 13. In five of these 13 the endocervical curettage showed microinvasion or invasion but in all five cases the colposcopic biopsy also showed microinvasion or invasion. Thus overall among 1042 patients who had an endocervical curettage done in only four did the endocervical curettage yield tissue that was much worse (greater than one grade) histologically than that found on colposcopic biopsy. Also of note is the absence of microinvasion or invasion diagnosed on the endocervical curettage and not on the colposcopic biopsy. On the basis of our data it appears that the endocervical curettage will very infrequently yield a more severe histologic diagnosis that will change management significantly. The endocervical curettage may more often modify management by simply identifying endocervical disease.

Visualization of the squamocolumnar junction and endocervical curettage are both used to identify the presence of endocervical disease and should correlate closely with each other. In those patients who had both endocervical curettage and visualization of the squamocolumnar junction in the 1977 to 1983 series these cross correlations are displayed in Table IV. The cross correlations are displayed for the entire series, for patients with carcinoma in situ, microinvasion, and invasion, and for patients with discrepant colposcopy–cone biopsy pathologic results. The squamocolumnar junction and endocervical curettage obviously do not perfectly cross correlate and one may suggest endocervical disease when the other does not. In the 14 patients with microinvasion or invasion who had both squamocolumnar junction and endocervical curettage evaluation, there were no patients with the squamocolumnar junction seen, but five of the 14 had a negative endocervical curettage result. Of note is that in the entire series, when the squamocolumnar junction was seen, the endocervical curettage result was positive in 7% and negative in 93%. When the squamocolumnar junction was not seen the endocervical curettage result was positive in 34% and negative in 66%. When the endocervical curettage result was positive the squamocolumnar junction was not seen in 62% and was seen in 38%. When the endocervical curettage result was negative the squamocolumnar junction was not seen in 20% and was seen in 80%. On comparison of all the cross correlations in Table IV, squamocolumnar junction visualization seems to be a more sensitive indicator of endocervical disease and of severe disease than the endocervical curettage result. Nevertheless the endocervical curettage occasionally reveals endocervical disease unsuspected by the squamocolumnar junction visualization and, when positive, is more often associated with severe disease than is nonvisualization of the squamocolumnar junction, as shown in Table V.

Exocervical lesion size as it relates to the severity of the ultimate pathologic diagnosis has also been analyzed. The size of the lesions was estimated by reviewing the diagrams of the lesions in the colposcopy logbook and then comparing lesion sizes for patients in the whole series with those of the patients with carcinoma in situ, microinvasion, and invasion. A small lesion was defined as no exocervical lesion or a lesion <8 mm^2. A large lesion was defined as an exocervical lesion >200

Table V. Association of microinvasion and invasion with squamocolumnar junction visualization, endocervical curettage result, lesion size, and age

	Entire series	*Microinvasion*	*Invasion*	*Chance of microinvasion or invasion (%)*
Squamocolumnar junction				
Seen	1623	6	0	0.37
Not seen	546	14	16	5.5
Endocervical curettage result				
Positive	154	3	10	8.4
Negative	888	2	3	0.56
Size of exocervical lesion				
Small (0-8 mm^2)	261	0	1	0.38
Medium (8-200 mm^2)	1524	15	13	1.8
Large (>200 mm^2)	259	8	5	5.0
Age (yr)				
15-18	190	1	0	0.53
19-20	348	0	0	0
21-25	717	5	2	0.98
26-30	350	5	0	1.4
31-40	241	3	4	2.9
41-50	100	3	3	6
>50	87	6	10	18
Entire series	2194	23	19	1.9

mm^2. Medium lesions were >8 and <200 mm^2. By this definition 13% of the entire group of 2044 patients without previous therapy had small lesions, 13% had large lesions, and 74% had medium lesions. Of 237 patients with carcinoma in situ 4% had small lesions, 19% had large lesions, and 78% had medium lesions. Of 42 patients with microinvasion or invasion 2% had small lesions, 31% had large lesions, and 67% had medium lesions. This demonstrates the increasing lesion size associated with more severe disease. Table V displays the correlation of lesion size with microinvasion-invasion. Particularly notable is the rarity of small lesions in microinvasive-invasive disease.

Increasing patient age is another important factor correlated with severity of pathologic results, nonvisualization of the squamocolumnar junction, and positive endocervical curettage results. The percentages of patients with carcinoma in situ, microinvasion, or invasion for each age group are: 15 to 20 years old, 3%; 21 to 25 years old, 8%; 26 to 30 years old, 13%; 31 to 40 years old, 23%; 41 to 50 years old, 25%; and over 50 years old, 39%. Age correlations with microinvasion-invasion are shown in Table V. The percentages of patients with positive endocervical curettage results for each age group are: 15 to 20 years old, 5%; 21 to 25 years old, 11%; 26 to 30 years old, 14%; 31 to 40 years old, 22%; 41 to 50 years old, 36%; and over 50 years old, 46%. The percentages of patients with nonvisualization of the squamocolumnar junction for each age group are: 15 to 20 years old, 7%; 21 to 25 years old, 15%; 26 to 30 years old, 20%; 31 to 40 years old, 37%; 41 to 50 years old, 81%; and over 50 years old, 90%.

Table VI. Endocervical curettage result and squamocolumnar junction evaluation in patients with microinvasion and invasion

	Series		
	1971-1976	*1977-1983*	
Microinvasion			
Squamocolumnar junction			
Seen	5/18	1/5	
Not seen	10/18	4/5	
Not indicated	2/18		
Previous hysterectomy	1/18		
Endocervical curettage result			
Positive	1/18	2/5	
Negative	0/18	2/5	
Not done	16/18	1/5	(pregnant)
Previous hysterectomy	1/18	–	
Invasion			
Squamocolumnar junction			
Seen	0/8	0/11	
Not seen	5/8	11/11	
Not indicated	2/8	0/11	
Previous hysterectomy	1/8	–	
Endocervical curettage result			
Positive	3/8	7/11	
Negative	0/8	3/11	
Not done	5/8	1/11	

Comment

Our analysis reveals that the colposcopy clinic was particularly successful in accurately identifying the 42 patients with microinvasive-invasive disease with absence of patients who were treated by cryotherapy and later found to have these more severe lesions. The important considerations in this outcome included eval-

uating the degree of Papanicolaou smear abnormality, the colposcopic biopsy pathologic results, the squamocolumnar junction visualization, and the use of cone biopsy and/or hysterectomy for the management of patients with severe dysplasia and carcinoma in situ. The endocervical curettage findings were less helpful. In the retrospective review it does not appear that the findings on endocervical curettage contributed significantly to the discovery of any of the 42 patients with microinvasion or invasion as none of the 18 of these patients who had an endocervical curettage had a positive result when the squamocolumnar junction was visualized. In this series severe dysplasia and carcinoma in situ were usually treated surgically regardless of endocervical evaluation. Cone biopsy in patients with severe dysplasia or carcinoma in situ on Papanicolaou smear or on colposcopic biopsy, even though the squamocolumnar junction was seen, led to the diagnosis of microinvasion in two of 23 patients with microinvasion. These patients were both seen before 1977, when endocervical curettage was not being done, and results of endocervical curettage may possibly have been positive in these two patients. However, our data would suggest that this probably would not have been the case. As displayed in Table IV, four patients later in the series who had microinvasion and who had both endocervical curettage and squamocolumnar junction evaluation included two with positive endocervical curettage results and two with negative results and the squamocolumnar junction was not seen in any of the four. All observers of the colposcopic approach have commented on the necessity of visualization of the entire lesion and the squamocolumnar junction if one is to rely on colposcopic biopsy to be most accurate in ruling out microinvasion and invasion.[1-10] Many have observed that even with squamocolumnar junction visualization the colposcopic biopsy does not totally exclude the presence of microinvasion and invasion (particularly the former) and our series confirms this observation.[1, 3-5, 7, 9]

Endocervical curettage has been considered by a number of observers and the usage varies from nonuse,[1, 2, 6] to use only when the squamocolumnar junction is not seen,[3, 4] to use only when it would contritube to the management,[10, 11] to use in all patients,[5, 12, 15] and some believe the results are always positive in patients with invasion.[12-14] Articles reviewing cases in which patients developed invasive disease after colposcopy and cryotherapy[15, 16] have led to the observation that endocervical curettage was often omitted in those with later invasion but these articles also reveal that endocervical curettage was done and the result was negative in a substantial number of the patients with later invasive disease. Many colposcopy series include a fairly small number of patients with microinvasion or invasion, but still attempts are made to arrive at conclusions regarding the role of squamocolumnar junction visualization and endocervical curettage in detecting these patients. Our series is larger than most but nevertheless we are reluctant to make conclusive statements about squamocolumnar junction visualization and endocervical curettage except to observe that neither is totally accurate in excluding microinvasion-invasion. Even when used in combination and in conjunction with the colposcopic biopsy they should not be expected to always exclude microinvasive-invasive disease. On the basis of our patients with microinvasion and the available literature it would appear that if one is to treat severe dysplasia–carcinoma in situ with cryotherapy then both squamocolumnar junction visualization and a negative endocervical curettage result should be present to exclude microinvasion-invasion to the maximal degree. On the basis of our data and those of others,[1-3] if one uses a surgical approach with more severe disease (severe dysplasia–carcinoma in situ) regardless of endocervical evaluation, then adding endocervical curettage evaluation to squamocolumnar junction evaluation for all patients does not appear to be of significant value. Others, however, suggest that endocervical curettage should be done even in those with only mild to moderate dysplasia on Papanicolaou smear and colposcopic biopsy and where the squamocolumnar junction is seen because it appears that even these patients will on rare occasion have a positive endocervical curettage result leading to the diagnosis of invasive disease.[7, 15]

Evaluation of the severity of the Papanicolaou smear and colposcopic biopsy results seem to be important correlates of the likelihood of associated microinvasion-invasion in our series. Some have implied that the severity of the disease on Papanicolaou smear or colposcopic biopsy short of microinvasion-invasion is not important in the selection of therapy as long as endocervical disease is ruled out by endocervical curettage and squamocolumnar junction visualization.[5, 17] Our data would not support this approach because of the strong correlation between Papanicolaou smear and colposcopic biopsy severity and ultimate diagnosis of microinvasion-invasion, plus the observation that squamocolumnar junction visualization and endocervical curettage will not always rule out microinvasion or invasion.

Exocervical lesion size has been correlated with the severity of the subsequent pathologic result by others,[18, 19] and it has been suggested that large lesion size may result in less accurate biopsies unless one samples the entire large lesion.[17-19] Our data are notable in revealing the particularly infrequent occurrence of small exocervical lesions in patients with microinvasion-invasion.

Age has long been correlated with an increasing incidence of many malignancies, and there is also an age correlation with severity of disease in the dysplasia–

carcinoma in situ–invasion continuum of cervical disease. This is demonstrated in our data as well as those of other observers.[2, 14] Increasing age is also correlated with squamocolumnar junction nonvisualization and positive endocervical curettage findings as documented by our data and those of others.[2, 14, 20] This appears related to the gradual migration of the squamocolumnar junction upward into the cervical canal because of the squamous metaplasia that occurs in all women. Notable is that these age correlations are infrequently incorporated into the management plans that have been proposed. It would seem reasonable that age should be integrated into the management plan as an important variable because of the increased likelihood of microinvasion-invasion in older patients as well as the existence of less concern in the older patient that conization or hysterectomy will interfere with childbearing.

The integration of these variables of Papanicolaou smear, colposcopic biopsy, squamocolumnar junction visualization, endocervical curettage, lesion size, and age into a management approach is somewhat complex because all are correlated with each other and all are predictive of the likelihood of finding microinvasion and invasion but none is perfect in that regard. Various possibilities in data integration will be the subject of a future report.

In summary a colposcopy clinic experience in evaluating 2194 patients with abnormal Papanicolaou smears is presented with a demonstration of the importance of various factors and of their multiple cross correlations.

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Editors' note: This manuscript was revised after these discussions were presented. The scoring system referred to in the discussion and the treatment and follow-up data were deleted from the revised manuscript.

Discussion

Dr. Maurice J. Mueller, Crescent Springs, Kentucky. The sheer numbers of patients involved in this study testify to its importance. Certainly the presence of 42 patients with microinvasion or invasive disease constitutes a wealth of experience. This article is especially helpful for the generalist not working in a clinic-type situation, who may not do this many colposcopies in an entire lifetime. It has been said that most invasive cancers occurring after conservative therapy are in those patients treated by less experienced colposcopists.[1] The use of the author's scoring system appears to be an excellent scheme for the nonexpert colposcopist.

The data here again prove that even milder Papanicolaou smear abnormalities may be associated with advanced disease. There were five patients with mild or moderate dysplasia on Papanicolaou smear who were shown to have microinvasion or invasion. This demonstrates the importance of evaluating all cases of dysplasia with colposcopy. Unfortunately, there are still some localities and some laboratories that choose to follow up these cases of milder dysplasia with repeat Papanicolaou smears only. Even though this association is not common, any delay in the diagnosis of advanced disease could obviously be disastrous.

Although not part of his scoring system, it would be interesting to know if the author considered the presence of an abnormal vascular pattern and its relation-

ship to advanced cervical disease. Sillman et al.,[2] in 1981, reported the occurrence of atypical vessels with invasive cervical cancer. Of their 60 patients with atypical vessels on colposcopy, all had either carcinoma in situ or invasive cancer. They felt this relationship to be great enough to recommend conization if the cervical biopsy did not show invasion.

This article includes endocervical curettage in the evaluation. Although the actual technique of endocervical curettage can vary greatly among individuals, I feel that it is important in order to get the maximum amount of information. In the landmark article of Townsend et al.,[3] in 1981, it was recommended that every patient with an abnormal smear undergo an endocervical curettage unless pregnant. Their review included 17 patients who did not have an endocervical curettage at the time of colposcopy, who then developed invasive cancer after cryotherapy. In a study by Hatch et al.,[4] in 1985, the endocervical curettage contained neoplastic epithelium in all 15 of the patients with invasive cancer, and in seven patients it was the only diagnostic parameter that indicated invasion.

It is significant that no patient in this present study had cryotherapy and was later found to have microinvasion or invasion. This indicates a well-thought-out triage procedure and a high degree of colposcopic skill and diligence. This is also borne out by five particular cases of microinvasion diagnosed by colposcopic biopsy. After conization or hysterectomy, these patients were found to have no residual microinvasion or invasion. Apparently the entire lesion had been removed by the biopsy in these cases.

The author admits that follow-up in the cryotherapy group was less than perfect. The follow-up problem is unfortunately quite common in large clinic populations. Of the 565 patients treated with cryotherapy, only 372 (66%) had a follow-up of at least one Papanicolaou smear. Of these 372, 101 patients had at least one follow-up Papanicolaou smear showing dysplasia. Thus there were 101 known cases of possible persistence of disease. Forty-seven of these 101 had subsequent biopsies, with 25 having dysplasia. The long-term follow-up of the 101 patients was not mentioned.

This very large series allowed the development of a quite practical regimen for the colposcopic evaluation of the abnormal Papanicolaou smear. With the multiple variables involved in technique, the Wetrich scoring system gives a more objective viewpoint. Application of this system should bring optimum results in the management of these patients.

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DR. KENNETH L. NOLLER, Rochester, Minnesota. I would like to congratulate Dr. Wetrich on his exhaustive efforts to document the experience of the University of Iowa Colposcopy Clinic for a period of 13 years. I would also like to thank him, as a clinician interested in colposcopy, for once again demonstrating to us that colposcopy is a safe and effective method for the evaluation and treatment of patients with cervical intraepithelial nneoplasia. It is indeed unfortunate that the short time interval allowed for presentation of papers at this meeting does not permit him to more fully develop the wealth of material that is found in the full manuscript that he has submitted. However, the area he has chosen to review with us today is certainly the most interesting and most controversial portion.

During the period of this study, 41 patients in whom the cervix was present were diagnosed as having either microinvasive or invasive squamous cell neoplasia of the cervix. All of these were identified as a part of the initial evaluation and none was found for the first time after outpatient therapy. I believe that there can be little doubt that colposcopy is the indicated procedure of choice following the report of an abnormal Papanicolaou smear.

In an attempt to increase the predictive value of the colposcopic images seen, Dr. Wetrich analyzed the factors that are commonly associated with invasive cancer. Similar to other investigators, he found that the severity of the Papanicolaou smear, the diagnosis obtained by colposcopic biopsy, the report of the endocervical curettage, the lesion size, and the age of the patient were all positive predictors of invasive disease. However, Dr. Wetrich then goes on to suggest that the "branching algorithm" approach, which is the basis for the colposcopic method, is outdated. He states: "This approach does not allow for proper integration of the available factors . . ." Based on this opinion, a scoring system was developed to aid in prediting which patient would have invasive cancer.

I believe that this is an improper conclusion based on the results of the study. Dr. Wetrich's study has shown that the method used during the study period, the "branching algorithm" approach does work. Table I shows how the usual approach to colposcopy was applied in the 16 evaluable cases of frank invasion that were discovered during this study. No invasive cancer was missed with this approach.

Table II shows the colposcopic technique applied to the 21 evaluable cases of microinvasion discovered during the series. It is not clear how one of the patients was identified. This case was in a 23-year-old patient with a Papanicolaou smear showing grade 3 cervical intraepithelial neoplasia, carcinoma in situ on the biopsy, a satisfactory examination, and a moderate lesion size. No endocervical curettage was performed. There-

Table I. University of Iowa colposcopy series: Invasion

Cases, 18	→ Incomplete records, 2	
↓		
Colposcopy, 16	→ Unsatisfactory examination, 16	
↓		
Satisfactory examination, 0	→ Colposcopic biopsy: invasion,	14
	microinvasion,	2
	cervical intraepithelial neoplasia,	3

Table II. University of Iowa colposcopy series: Microinvasion

Cases, 23 → incomplete records, 2
↓
Colposcopy, 21 → unsatisfactory examination, 14 → conization
↓
Satisfactory examination, 7 → microinvasion on biopsy or examination, 11 → conization
↓
Satisfactory biopsy, 2 → disparity between Papanicolaou smear and biopsy, 1 → conization
↓
Endocervical curettage, 1 → not done

fore this is an unevaluable case since it is quite likely that an endocervical curettage would have picked up the small microinvasive cancer that was found in the conization specimen.

The proposal for a scoring system is, in my opinion, unwarranted. This large study has failed to show a single case of invasive cancer in which the diagnosis was missed. This laudable record was achieved with the old system. Why then change to a new system?

The graphs shown by Dr. Wetrich demonstrate a bimodal distribution of point values for the cases seen with carcinoma in situ and severe dysplasia. This suggests that the system adds nothing to the usual colposcopic technique. In fact the distribution, as demonstrated by the graphs, argues against the use of the system. The first peak on the graph is a result of the high value assigned to lesions of grade 3 cervical intraepithelial neoplasia or worse and the second peak is due to nonvisualization of the squamocolumnar junction or a positive endocervical curettage result. There appears to be little interaction of these variables.

Additionally, with the scoring system known, it is possible to "invent" patients who should probably have conization but do not receive enough points to require conization. For example, a 21-year-old woman with mild to moderate dysplasia and a small lesion with a nonvisualized transformation zone would have a total of only 16 points by the proposed scoring system. This would make her a candidate for cryocautery even though the examination was unsatisfactory. Likewise, an 18-year-old woman with mild to moderate dysplasia and a positive endocervical curettage result with a small lesion would also be a candidate for outpatient therapy. Neither of these patients fulfills the accepted criteria for outpatient therapy. Although these patients are totally fictitious, they are not unrealistic, and it is certainly possible that such women would eventually present at the clinic and be mismanaged if the scoring system were strictly applied. However, the "branching algorithm" approach would correctly require conization in both cases.

I would like to ask Dr. Wetrich two questions: First, is the scoring system used at the University of Iowa Colposcopy Clinic at the present time? Second, if the system is presently being used, would the two fictitious cases that I have just proposed be treated by outpatient cryocautery in the face of an unsatisfactory examination or a positive endocervical curettage result?

In conclusion, I would like once again to congratulate Dr. Wetrich on an excellent piece of work documenting the usefulness of colposcopy. While he and I differ slightly on the conclusions that should be drawn from the study, this article is a most important addition to our current approach toward the correct diagnosis and management of patients with cervical intraepithelial neoplasia.

Dr. D. Foley, Milwaukee, Wisconsin. In the five patients with a Papanicolaou smear showing mild dysplasia, did the repeat Papanicolaou smear still show mild dysplasia?

Dr. Wetrich (Closing). Dr. Mueller asked about the colposcopic appearance of the lesion as a possible variable that we might incorporate into our management. As anyone who has done colposcopy knows, we think

we can somewhat predict the severity of the lesion by its appearance. We have not really made any attempt to integrate that into our plan of management because it seems to us that ultimately we depend on the biopsy, regardless of what the lesion looks like, so it would not be too helpful to add that into our management plan. Certainly one does get impressions, but I guess if a lesion looked like invasive cancer and the biopsy results did not show that, I would not change my management just based on the appearance.

Dr. Mueller also commented on the fact that our follow-up on patients who underwent cryotherapy was not ideal, and I would agree with him. I commented on that in the full manuscript. About one third of our cryotherapy patients were not followed up at our institution. In our institutional setting many patients come from some distance away, and in many cases it is much more convenient for them to have follow-up through their local physician or local clinic. We do not insist that they return to University Hospitals for routine follow-up after cryotherapy.

It certainly is possible that some of those patients did have microinvasion or invasion that we did not know about. We do know that many of them are referred back to us if they have recurrent abnormalities after cryotherapy, and we would like to think that if they had microinvasion-invasion they would also be referred back to us; but certainly that is an appropriate criticism.

Dr. Noller presented an analysis of our case list of patients with microinvasion-invasion and what might have happened in those patients if one used a fairly simple branching algorithm approach depending primarily on the presence or absence of endocervical disease. It is true that all of our cases of invasion would have been detected with this approach. On the other hand, in two of our patients, microinvasion might have been missed with this approach. In both patients the squamocolumnar junction was visualized and a colposcopic biopsy specimen did not show microinvasion. These patients were from the early part of our series when we were not doing endocervical curettage. It is possible that the endocervical curettage findings might have been positive in these patients had the procedure been done. However, only 7% of our patients who had visualization of the squamocolumnar junction had a positive endocervical curettage result, so these patients probably would not have had a positive finding at curettage. There may be a certain amount of conjecture in that, but it seems like a reasonable conclusion.

In addition to our data, it should be noted that there are numerous other series in the literature at the present time that document cases of microinvasion not diagnosed on colposcopic biopsy even though the colposcopy was satisfactory, with the squamocolumnar junction seen. Also data in the literature demonstrate a number of patients with microinvasion or invasion who had negative endocervical curettage results, as was found in our series. So, on the basis of our data and other published data, it seems that total reliance on endocervical disease evaluation as the only indication for cone biopsy may be inappropriate. We would like to think that adding in the additional factors might lead to a more reasonable approach.

Dr. Noller commented that perhaps a more formalized statistical multivariant analysis would be appropriate. I think that is an excellent idea, and I intend to pursue it.

Dr. Noller also gave a couple of hypothetical examples of patients who were borderline cases and questioned whether that was really the appropriate way to manage the patients. Both of these patients had mild to moderate dysplasia and were also in the very young age group and had small lesions. My only comment on that would be that in our series of patients, in all those who had less than moderate dysplasia (including mild or mild to moderate dysplasia) as the worst abnormality on Papanicolaou smear, colposcopic biopsy, or endocervical curettage, there were no cases of microinvasive or invasive cancer; a series of about 700 patients would fit this description. Dr. Noller is picking a borderline situation that people might differ with, but I think the recommendation of the scoring system would not be far off base.

Dr. Noller also asked about the use of the scoring system in our clinic. Perhaps I should comment a bit more about the clinic. Specifically, I myself, in conjunction with the residents in training, did all the initial colposcopic evaluations of these patients. After colposcopic evaluation, their management was then the responsibility of other staff physicians, and during the course of the study about 10 different staff gynecologists were involved in making the postcolposcopy decisions. Obviously this is not going to lead to an entirely consistent approach, because everyone has different ideas about management, so we did not have any strict protocol on postcolposcopy management.

Almost all of the patients with carcinoma in situ did have cone biopsy regardless of endocervical involvement. A great majority of patients with severe dysplasia also had cone biopsy regardless of the endocervical evaluation. In addition, in patients with a milder disease a variety of things were done. Women who had mild dysplasia without endicervical disease were of course almost all treated with cryotherapy, but if they had endocervical disease a number of things were done. Some were just followed up with Papanicolaou smears, some had cryotherapy, and some had cone biopsy, so there was not really a very consistent approach to these patients with mild disease.

We have not had any strict protocol in terms of how these cases have been managed. Right now we are going through a transition in the way the clinic operates. We hope to have a more systematic approach and to be able to incorporate the principles of the scoring system.

Dr. Foley had a question about the patients with mild disease on Papanicolaou smear. I think he was referring to patients who had mild dysplasia and who were found to have microinvasive or invasive disease. In the data given here, only the Papanicolaou smear taken at the colposcopy clinic is included. Obviously these patients

had Papanicolaou smears taken before being referred to the colposcopy clinic, and some had Papanicolaou smears after that. I do not have a detailed breakdown of data to answer his question, but in some cases, yes, the additional Papanicolaou smears showed more than mild dysplasia. I cannot give you a more detailed breakdown on that specific question, but in some cases the patients had worse than mild dysplasia on subsequent or prior Papanicolaou smears.

Chorionic villus sampling in continuing pregnancies

I. Low fetal loss rates in initial 109 cases

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Among the first 150 women who agreed to have chorionic villus sampling after receiving counseling and giving informed consent, 41 proved ineligible. In six (5.5%) of the remaining 109 cases in which chorionic villus sampling was performed, we were unsuccessful in obtaining an adequate amount of villi to permit diagnostic testing. In the single loss, fetal viability was confirmed 2 weeks after sampling; however, fetal death became evident 3 weeks later. In four (3.7%) cases the pregnancies were terminated because of abnormal results, and in one (0.9%) case the pregnancy was electively terminated after normal results. Among the 41 completed pregnancies no anomalies were evident in the infants. There were two premature deliveries; one of these two infants died shortly after birth following premature rupture of the membranes at 29 weeks' gestation. All undelivered cases were progressing normally at the time of submission. (AM J OBSTET GYNECOL 1986;154:1349-52.)

Key words: Chorionic villus sampling safety

At the 1984 annual meeting of The Central Association of Obstetricians and Gynecologists we presented our initial experience in developing a chorionic villus sampling program at Northwestern University.[1] At that time we stated the following: "Chorionic villus sampling for first-trimester prenatal diagnosis has the potential of becoming an attractive alternative to genetic amniocentesis. Prior to becoming a standard, however, considerable caution and monitoring is necessary to establish the accuracy and safety of the procedure." In this pursuit, we wish to present our continued experience in the technical aspects and safety of chorionic villus sampling. In our companion paper,[2] we present our results with respect to cytogenetic reliability and accuracy.

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Material and methods

In August 1983 we initiated our chorionic villus sampling program at Northwestern University by developing surgical and laboratory experience in a series of 58 volunteers undergoing elective first-trimester abortion (6 to 12 weeks' gestation).[1] Our initial experience was sufficiently encouraging that in March 1984 we sought and obtained approval from the Institutional Review Board of Northwestern University to offer chorionic villus sampling in continuing pregnancies. The following were considered indications for the procedure: (1) advanced maternal age (usually age ≥35 years; (2) previous trisomic offspring; (3) parent with a balanced structural chromosomal rearrangement; and (4) fetus at high risk for a detectable mendelian disorder (for example, Tay-Sachs disease, sickle cell disease).

A preliminary ultrasound examination was performed on all patients fulfilling these criteria who were

Table I. Screening for chorionic villus sampling

Number of patients screened	
Subjects excluded	188
Declined after counseling	38
Advanced gestational age (>11.3 weeks)	12
Fetal death (missed abortion)	15
Twins	3
Active herpes cervicitis	1
Severe vulvovaginitis *(Candida albicans)*	1
Threatened abortion (uterine bleeding)	3
Markedly retroverted and retroflexed uterus (posterior placenta)	3
Inaccessible fundal placenta	1
Leiomyomas	2
Patients undergoing chorionic villus sampling	109

Table II. Indications for chorionic villus sampling (n = 109)

Maternal age >35 years	96
Maternal age <35 years	4
Previous trisomic offspring	
+21, live-born	2
+13, live-born	1
+13, chorionic villus sampling	1
+21, amniocentesis	3
Translocation heterozygote—45,XX,−13,−13,+t(13q;13q)	1
Niemann-Pick disease (two previous offspring)	1
Total	109

interested in chorionic villus sampling. Only patients whose pregnancies were between 7 and 11 weeks' gestation, based on onset of the last menses and confirmed by ultrasonographic measurement of the fetal crown-rump length (9 to 41 mm) remained eligible. Patients were excluded if (1) fetal viability was not confirmed by ultrasonographic visualization of fetal heart motion; (2) multiple gestation was detected; (3) pathologic conditions coexisted that conferred increased risk in performing chorionic villus sampling (for example, active genital herpes simplex infection, cervical pathologic findings, severe cervicitis, undiagnosed cervical lesion, active uterine bleeding); (4) large submucous uterine leiomyomas precluded reasonable access to the chorionic villi; (5) a markedly angulated cervical canal precluded ready catheter passage; and (6) the placenta was fundal and deemed inaccessible. (With increasing operator experience this last exclusion criterion became less applicable.) All eligible patients underwent formal genetic counseling and gave informed consent for chorionic villus sampling.

We basically continue to perform the procedure, as previously described,[1] in an ambulatory surgical suite to minimize the risk of infection. We use a 2.1 mm outer-diameter radiopaque Teflon catheter (E-Z Cath, Deseret Pharmaceutical Co., Inc., Sandy, Utah), trimmed by scissors to fit snugly over a blunt-ended aluminum stylet. Use of the catheter for this purpose is under an investigational device exemption approval from the Food and Drug Administration. If additional attempts are required to obtain an adequate specimen, a fresh catheter is used each time.

A real-time mechanical sector transducer (3.5 MHz) is employed to visualize the uterus and to guide the catheter into the optimal site of the placental substance. After aspiration, villi are transferred to a Petri dish containing 5 ml of modified Ham's F-12 media and inspected through a dissecting microscope (10×). The quantity of tissue is judged to be adequate if at least 5 mg of villi were obtained. Optimal samples weigh 15 to 20 mg. The quantity of tissue is estimated by comparison to a predetermined reference standard in which varying amounts of villi were weighed and photographed. Specimens are immediately transported to our laboratory for processing. The cytogenetic methods we are currently using are provided in our companion paper.

Following this procedure, fetal heart movements are verified by ultrasonographic visualization, and the patient is monitored for at least 30 minutes for any untoward effects. (None has been observed.) Patients at risk for Rhesus isoimmunization receive 300 μg of anti-D immune globulin if their indirect Coombs' test is negative.

Patients are also routinely contacted by telephone 3 days following the procedure. At 15 weeks' gestation an ultrasound examination is performed to detect overt fetal malformations and to assess fetal growth. Maternal serum α-fetoprotein is also determined.

Because maternal cell contamination in the chorionic villus sampling specimen could lead to an erroneous fetal diagnosis, our investigative protocol provides the option for patients with a 46,XX complement to have a subsequent confirmatory genetic amniocentesis at no additional cost.

Results

Among 188 women screened for chorionic villus sampling, 41 were considered ineligible, and 38 declined the procedure (Table I). The indications for prenatal diagnosis in those 109 women who underwent the procedure are summarized in Table II. Sampling was usually carried out at the ninth or tenth week (Table III). In six of the 109 (5.5%) cases we were unsuccessful at obtaining an adequate amount of villi to permit diagnostic testing. All six subsequently underwent successful amniocentesis. Table IV correlates the number of transcervical catheter passes correlated with the success or failure of obtaining a specimen adequate for analysis. The overall success rate was 94.5%.

Of the 103 successful samplings, 97 were cytogenetically normal: 46,XX (50); 46,XY (47). In six cases a

Table III. Gestational age at time of chorionic villus sampling

	*Weeks' gestation**				
	7 (49-55 days)	*8 (56-62 days)*	*9 (63-69 days)*	*10 (70-76 days)*	*11 (77-80 days)*
No. of subjects	1	5	55	41	7

*Assigned gestational age was the best estimate based upon the onset of the last menses correlated with ultrasonographic measurement of the fetal crown-rump length.

Table IV. Correlation between number of transcervical passes required for successful chorionic villus sampling and the time during program at which sample was attempted (operator success improved with experience)

Order of cases	*Number of attempts*						
	1	*2*	*3*	*4*	*5*	*6*	*Failed*
1-20	3	7	4	2	2		2
21-40	13	4	3				
41-60	15	3				1	1
61-80	13	5	1				1
81-100	11	6	1				2
100-109	7	1	1				
Total	62	26	10	2	2	1	6

chromosomal abnormality was detected from the chorionic villus sampling specimen, as will be discussed in our companion paper. In one of our early cases (No. 8) the chorionic villus specimen showed a 45,XX complement, but the infant was male. Analysis was based on three cultures; no metaphases from the direct method were available. This finding suggested maternal cell contamination. The pregnancy at risk for Niemann-Pick disease was determined to be unaffected (biochemical assay performed by Dr. David Wenger, Denver, Colorado). This pregnancy was still undelivered at the time of submission.

Pregnancy outcomes of all 109 women undergoing chorionic villus sampling are summarized in Table V. Only one fetal loss has occurred, that being detected 5 weeks following the procedure. An ultrasound scan was normal 2 weeks after chorionic villus sampling, indicating that fetal death occurred 2 to 5 weeks after the sampling. In this case, two catheter passes were required to obtain an adequate sample that subsequently proved to be 46,XX. The patient experienced intermittent vaginal spotting during the first week after the procedure, but fetal heart tones were confirmed by Doppler ultrasound 2 weeks later. However, fetal heart tones were absent 5 weeks after chorionic villus sampling; fetal death was confirmed by ultrasonographic examination.

In two cases several milliliters of clear yellow fluid was inadvertently aspirated from the catheter during the initial passage, and the procedures were discontinued. However, in neither case did the gestational sac collapse, and in each fetal heart motion was normal. Both patients were followed with serial ultrasonographic examinations, with no evidence of oligohydramnios, leakage of fluid, or infection. The first patient underwent subsequent amniocentesis at 16 weeks, which showed normal 46,XY complement. This patient has been delivered of a healthy 4665 gm infant. The second patient also underwent amniocentesis with results pending at the time of submission; this pregnancy is progressing normally.

Table V. Pregnancy outcomes of the 109 women undergoing chorionic villus sampling

	n	%
Number of patients sampled	109	
Unsuccessful samplings	6	5.5
Fetal losses	1	.9
Terminated because of abnormal results	4	3.7
Elective abortion after normal results	1	.9
Delivered at term	41	
Delivered prematurely*	2	
Continuing pregnancies	54	

*In one case delivery was at 34 weeks with normal outcome; in the second case neonatal death occurred at 29 weeks.

Comment

Although still investigational, chorionic villus sampling is already welcomed as an important advance in prenatal diagnosis. However, despite worldwide experience with more than 7000 procedures,[3] precise data with respect to safety are not yet available. We have

previously addressed the complexities of attempting to assess the safety of chorionic villus sampling.[1-4] Suffice it to repeat that the rate of 4% to 5% fetal losses observed worldwide after chorionic villus sampling is an absolute rate that must be corrected for a host of confounding variables: (1) accuracies in the identification and timing of spontaneous fetal loss rates, (2) maternal history of prior induced abortions, (3) parental cytogenetic factors (recurrent aneuploidy, translocations, inversions), (4) maternal age, (5) gravidity, (6) smoking, (7) alcohol consumption, and (8) environmental factors (for example, exposure to infectious agents, drugs, or chemical toxins).

In this context our own initial experience is still most encouraging. The overall success rate of obtaining a specimen adequate for analysis was 94.5%, but in 56.9% of samplings an adequate specimen was obtained with a single catheter pass. In an additional 23.9% of cases, two catheter passes were required; 13.8% of cases required three or more passes; in 5.5% we were unsuccessful. Only one fetal loss occurred among our first 109 cases, and this took place at least 2 weeks after the procedure. The fetus was determined to be chromosomally normal, and no cause could be documented for the loss.

Of special interest are the two cases in which fluid was inadvertently aspirated during chorionic villus sampling. In each, expectant obstetric management was successfully followed. Although the exact origin of the aspirated fluid could not be determined, failure of the gestational sac to collapse suggests to us that the fluid could have been derived from the space between the amnion and chorion. Alternatively, early amniocentesis may not necessarily be deleterious. In neither case was there evidence of oligohydramnios, transcervical fluid leakage, or infection. If there is documentation of an intact gestational sac by ultrasound, we now believe that expectant management is a reasonable option provided (1) couples are informed of potential maternal and fetal risk (for example, oligohydramnios, infection, spontaneous abortion); and (2) close fetal and maternal surveillance is possible to monitor indices of developing infection, fetal growth, and amniotic fluid volume. On the other hand, we would predict the risk of intrauterine or deformation infection to be great if the gestational sac is observed to collapse at the time of chorionic villus sampling. If such a pregnancy were allowed to continue, the risk of continued amniotic fluid leakage and oligohydramnios leading to fetal deformations would be substantial.[5] Accordingly, we would recommend that pregnancy termination be seriously considered in such cases.

Our overall low complication rate following chorionic villus sampling could be attributed to various factors. First, it could merely reflect the vicissitudes of yet relatively small numbers. However, we believe that our low total fetal loss rate (0.9%) in the first 109 cases may be compared favorably with the 2.0% to 4.4% absolute loss rates reported to the International Fetal Diagnosis Registry by the four most experienced centers worldwide.[3] Of course, we again emphasize that neither their data nor ours take into account the background fetal loss rates, nor are they corrected for the numerous confounding variables alluded to earlier. Best estimates at this time suggest that *procedurally related* losses will be in the range of 1% to 2%. Second, our sample (that is, primarily upper socioeconomic, white) could have been selected from a group of women less likely to undergo complications than other groups. Differences in nutritional status, number of sexual partners, parity, and other factors could exist. Third, our experience in performing chorionic villus sampling in a large series of women immediately before elective abortions could have permitted the two obstetricians performing the procedure to become adept with its technical nuances. Fourth, our conservative approach in not even attempting chorionic villus sampling in certain cases (for example, multiple gestation, large uterine leiomyomas, cervicitis, spotting) may have reduced the potential for complications. Fifth, the risk for infection could have been minimized by performing chorionic villus sampling in an ambulatory surgical suite and in using a new catheter for each attempt at obtaining villi.

In conclusion, we remain optimistic that chorionic villus sampling will eventually become a standard diagnostic technique for first-trimester prenatal diagnosis. At present, however, it must still be regarded as investigational and not yet an accepted alternative to midtrimester amniocentesis. More definitive data comparing risks and accuracy of chorionic villus sampling as compared to amniocentesis will have to await data from collaborative studies under way in the United States, Canada, and Europe.

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Discussion of part I and part II begins on page 1358.

Chorionic villus sampling in continuing pregnancies

II. Cytogenetic reliability

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Cytogenetic analysis was performed on 103 chorionic villus samples. Analysis of the 103 samples revealed six abnormalities. In three of the six the abnormalities were confirmed in fetal or neonatal tissue (47,XY, +13; 46,XY, t(13q13q); 45,X). In three samples the abnormalities detected were not confirmed; in two of the three the abnormalities were detected only in long-term cultures, whereas in the other samples the abnormality was restricted to direct analysis of the villi after overnight incubation. Our initial experience leads us to conclude that certain abnormalities in chorionic villus sampling may not be indicative of fetal abnormalities; 45,X/46,XX or 45,X/46,XY mosaicism is such a complement. Discrepancies between cytogenetic analysis of intact villi processed soon after sampling and of cells grown in culture can be managed by adhering to several suggested guidelines and by liberal use of confirmatory amniocentesis. (Am J Obstet Gynecol 1986; 154:1353-62.)

Key words: Chorionic villus sampling, cytogenetic reliability

Introduction of chorionic villus sampling as a routine diagnostic service not only requires confirmation of its safety but also its diagnostic accuracy. In our companion paper[1] we consider surgical techniques and safety of chorionic villus sampling. In this contribution we restrict our remarks to cytogenetic techniques and diagnostic accuracy in those 103 cases in which specimens were obtained. We propose guidelines for handling discrepancies between analysis of intact villi processed after overnight incubation and of cells derived from the villi and grown in tissue culture.

Material and methods

General approach. Since initiation of our chorionic villus sampling program, laboratory methods have evolved and expanded to improve quality of cytogenetic preparations, to increase efficiency, and to maximize diagnostic accuracy. Our initial methods have been described previously.[2] We continue to use two types of preparations: (1) direct analysis of mitoses present in intact chorionic villi, these cells presumed to have originated from budding cytotrophoblasts, and (2) analysis of cultured cells, the cultures having been initiated from mesenchyme cores of villi. We have performed cytogenetic analysis of cultured cells on all 103 subjects. "Direct" preparations were also successful in 79 cases. In some earlier patients analysis of intact villi was not always possible because (1) insufficient villi were obtained to attempt both types of preparations or (2) quality of the direct preparations was too poor to warrant analyses. However, only once among our last 59 specimens were both direct and culture methods not available.

Our current protocol is to visualize the specimen immediately after it has been obtained, with use of a dissecting microscope transported to the operating suite. We seek to determine whether at least 5 mg of villi have been obtained. If not, the obstetrician may elect to perform up to two additional aspiration(s). The specimen(s) is then transported to the laboratory in a 100 mm Petri dish containing 15 ml nutrient media, usually Ham's F-12 supplemented with heparin, penicillin, and streptomycin. The specimen is further examined under a microscope (10×) located in a horizontal flow hood (Edgeguard). If the specimen is extremely bloody, it is filtered through a 115 ml, 5 μ millipore filter unit (Nalgene Type A) before dissection. An estimate of the amount of material obtained is made by comparison with photographs of previously weighed villi (Fig. 1). Villi are then identified, dissected from nonvillus-appearing material, and washed in Hanks basic salt solution containing penicillin and streptomycin.

If the total specimen is <5 mg, we may elect to proceed with a culture only, bypassing the direct method.

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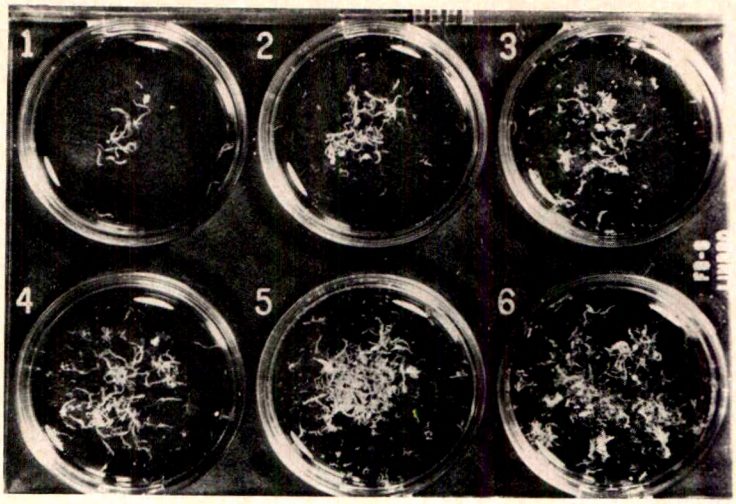


Fig. 1. Amount of dispersed chorionic villi in 5 to 40 mg aliquots.

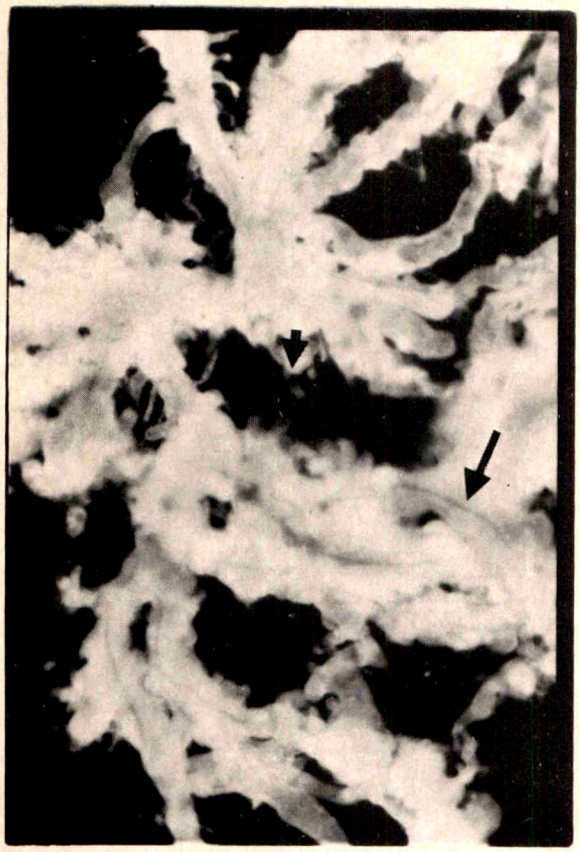


Fig. 2. Portion of chorionic villi showing buds *(short arrow)* and capillaries *(long arrow)*.

If both methods are to be attempted, villi are divided approximately equally for direct and culture preparations. The optimal-looking pieces (that is, villi with buds and capillaries) are allocated for the direct method.

Preparation of intact villi for analysis (direct method). Villi with buds and capillaries (Fig. 2) are necessary for the direct preparations. Such villi are placed in 2 ml of Chang's medium and incubated overnight at 37° C in an open system (5% carbon dioxide in balanced air). Specimens can also be processed immediately, but the mitotic index seems to increase when processed following overnight incubation (24 hours). Colcemid (final concentration, 0.15 μg/ml) is added for at least 3 hours. The medium is then aspirated, and 2 ml of 0.8% sodium citrate warmed to 37° C added. The specimen is left at room temperature for 20 minutes, following which the hypotonic solution is replaced with 2 ml of freshly prepared and chilled fixative (5 methanol/2 glacial acetic acid), and then chilled for 15 minutes at 4° C. This fixation is then repeated twice. The fixative is next aspirated, and the villi are allowed to air dry. They are then covered for 5 minutes with a few drops of 60% glacial acetic acid in distilled water. A small amount of the previously described 5 : 2 fixative is then added, the villi are vigorously aspirated up and down the pipette, and the suspension transferred to microscope slides.

Culture initiation. To initiate cultures, we first clean villi as already described. Photographs of villi are taken to document morphology of specimens used to initiate a given culture (A, B, C, etc.). This information will be used to determine (1) appearance of tissue in preparations in which maternal contamination is later demonstrated; (2) appearance of villi when a chromosomally abnormal complement is detected; (3) relationship between (a) villi appearance and quantity and (b) growth rate and quality of preparations. Two types of cultures are then prepared. In one type we use only prolonged trypsinization, whereas in the other we treat villi with trypsin ethylenediaminetetraacetate (EDTA) followed by collagenase. If only a few villi are available, the latter type is preferentiallly initiated because of the shorter time until analysis can proceed.

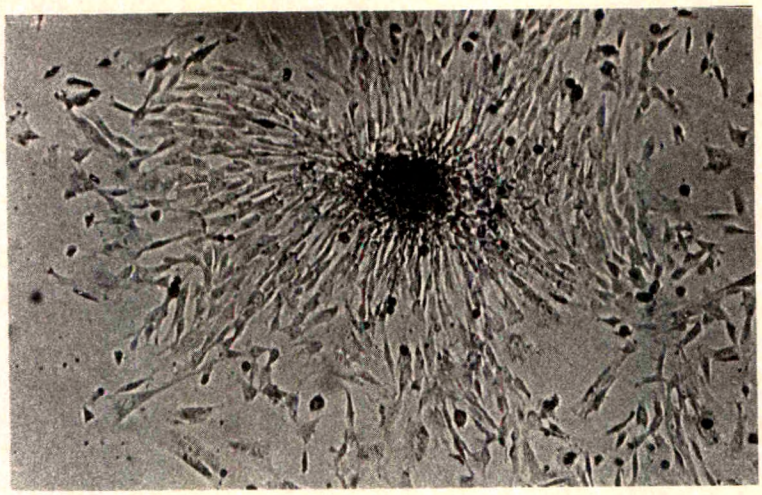


Fig. 3. A growth center in a culture derived from chorionic villi mesenchyme.

Table I. Indications and results

	No.	*46,XX*	*46,XY*	*Abnormalities*
Maternal age >35 years	91	48	43	45,X 47,XY,+13 46,XY/47,XY,+15* 45,XY,t(4;15)† 45,X/46,XX
Maternal age <35 years	4		4	
Previous trisomic offspring (two live-born, three amniocentesis, 1 chorionic villus sampling)	6	4	2	
Translocation heterozygosity—45,XX,t(13q13q)	1	1		46,XX,t(13q13q)
Niemann-Pick disease	1		1	

*Mosaicism was found in only direct cultures. Normal male fetus confirmed on amniocentesis.

†45,XY,t(4;15),(4qter→4p15 :: 5q→ 15qter. Because of amniotic sac puncture, only one small piece of villus was obtained. These results are from one culture.

Trypsinization culture method. After dissection and cleaning, villi are placed in a 60 mm Petri dish containing 3 ml of 0.25% trypsin. The dish is placed on a agitator for 2 hours. If villi are large and multibranched, the trypsinized villi are then filtered through a wire mesh. If pieces are small or individually identified, they are merely "swished" in the trypsin to remove debris adhering to the mesenchyme core. In either case a further 30-minute exposure to trypsin is employed, again with the Petri dish placed on the agitator. Pieces of villi are then washed in several changes of Chang's medium (open or closed system types) and placed into flaskettes (Lab Tek).

Trypsin-EDTA-collagenase culture method. After dissection and cleaning as previously described, villi are placed in 3 ml of trypsin-EDTA for 2 hours. Pieces of villi are then swished and washed to remove debris and placed in a 60 mm Petri dish with 5 ml of Chang's medium and 1 mg/ml of collagenase (Sigma Type V, 410 U/mg) overnight (13 to 17 hours). The suspension is then centrifuged (1000 rpm for 10 minutes), washed with supplemented medium (Chang's or minimal essential medium), and plated in flaskettes (Lab-Tek).

Subsequent culture and harvest. Irrespective of whether the trypsinization method or the combined enzyme method is used, cells usually attach in 1 to 2 days. Growth centers (Fig. 3) are classified as C (chorionic villus type cells, which are morphologically similar to "AF" cells observed in amniotic fluid cultures); E2 (epithelial-type cells, but cells which are *not* similar to epithelial cells characteristically observed in amniotic fluid cultures); F (fibroblast-like cells)[3]; or other. Incidentally, chorionic villus growth centers appear different from those observed in amniotic fluid cultures. The former show well-defined, central aggregates of cells surrounded by peripherally growing cells.

Cultures are monitored daily until the mitotic rate is sufficiently high to warrant harvest. The first cultures are ready for analysis at about 5 days and are those prepared by the trypsin-EDTA-collagenase method. Harvest is generally performed in situ on primary cultures, that is, enzymes are not used to disperse cells

Table II. Chorionic villus sampling abnormalities

	Direct preparation	*Culture*	*Confirmation*
47,XY,+13	UA	+	Yes (abortus)
45,X/46,XX	UA	+*	No (abortus)
46,XY,t(13q;13q)	+	+	Yes (abortus)
45,X	+	+	Yes (abortus)
45,XY,t(14p;15q) (unbalanced)	UA	+	No (amniocentesis)
46,XY/47,XY,+15	+	−	No (amniocentesis)

UA = Unavailable or unanalyzable.

*Fifteen 45,X cells were obtained from two cultures said to be initiated with villi. Sixteen 46,XX cells were obtained from two cultures with less optimal specimens at initiation. Consequently it was assumed likely that the sixteen 46,XX cells were maternal in origin.

Table IIIA. Incidence of aneuploidy in chorionic villus sampling according to maternal age (all gestational weeks)

	Abnormalities			
	Autosomes		*Sex chromosomes*	
Week	*n*	%	*n*	%
35-39	21/625	3.4	8/625	1.3
>39	21/271	7.6	4/271	1.5

*European Collaborative Data, 1984, from Mikkelsen.[7]

from their primary growth centers. However, cultures may be subcultured or treated with enzymes to disperse cells within the flaskette (1) if only a single culture is available, (2) if large numbers of metaphases are required for analysis, or (3) if especially high-quality karyotypes are needed.

Culture harvest then proceeds. We use 0.7% of sodium citrate for 20 minutes as a hypotonic solution, followed by four changes of methanol/glacial acetic acid (5:2) fixative. Microscopic slides are prepared from the suspension-containing cells and are aged at least overnight before being treated with pancreatin and stained with Wright's Giemsa stain.

Cytogenetic analysis. Ideally, 20 cells are analyzed from direct preparations and 20 from cultures. We especially prefer this number if a normal female chromosomal complement is encountered. If the complement is male, only 10 cells from each are counted. If mosaicism is detected, 50 to 100 cells are analyzed.

Results

Cytogenetic analyses have been completed for 103 patients with continuing pregnancies (Table I). Ninety-seven were diagnosed normal: 47 male (46,XY) and 50 female (46,XX). However, there was one instance

Table IIIB. Incidence of aneuploidy in chorionic villus sampling according to gestational age (all maternal ages)*

	Abnormalities	
Week	*n*	%
6-7	2/19	10.5
8	15/145	10.3
9	29/516	5.6
10	17/465	3.7
11	6/185	3.2
12-13	6/169	8.7

*European Collaborative Data, 1984, from Mikkelsen.[7]

of maternal cell contamination leading to a misdiagnosis of sex. There were six abnormal complements (Table II).

Of the 97 normal complements, eight were confirmed by cytogenetic analysis of amniotic fluid cultures. (Amniocentesis is offered to all women whose chorionic villus sampling complement is 46,XX). An additional 35 diagnoses were confirmed at birth with respect to sex and/or normal phenotypes. One normal pregnancy was electively terminated, and 54 pregnancies are still in progress.

The status of the six abnormal complements (Table II) is as follows: (1) The 47,XY,+13 case was confirmed on an abortus specimen, and thus was considered as validated. (2) The 45,X/46,XX mosaic was *not* confirmed on abortus material that was definitely fetal in origin; all 100 abortus cells were 46,XX. (3) The 45,XY,t(4;15)(4qter→4p15::15q15→15qter) unbalanced complement was confirmed at neither amniocentesis nor delivery (46,XY). A phenotypically normal male infant was delivered. This was not actually unexpected, since the initial abnormal complement was obtained from only one growth center in culture. This finding had thus been interpreted as a culture artifact, clonal in origin. The paucity of analyzable material occurred because only about 0.5 mg of villi were obtained, and these in very small fragments. Consequently, only one culture could be initiated, and only one center was analyzable. (4) The 46,XX,−13,+t(13q13q) complement was confirmed on abortus tissue. (5) The 46,XY/47,XY,+15 mosaicism was not confirmed at amniocentesis. The pregnancy continued, and a normal male infant was delivered. However, blood for cytogenetic confirmation has not yet been obtained. Interestingly, this mosaicism was confined to direct preparations. (6) A 45,X complement was observed in both direct preparations and in cultured cells. The same complement was confirmed in abortus material.

Overall then, we consider three abnormalities confirmed (Nos. 1, 4, and 6) and three not indicative of fetal anomalies (Nos. 2, 3, and 5).

Comment

The feasibility of routine cytogenetic evaluation of chorionic villi requires affirmative response to each of two general questions: (1) Do villi reflect fetal status, as opposed to being unrepresentative as the result of different embryonic origin? (2) Are villi obtained by aspiration of sufficient quality and quantity to render chromosomal analysis practical in a clinical setting? Early experiences of ourselves and others indicate that affirmative responses to both queries are likely. Notwithstanding our general optimism, however, several dilemmas must be resolved before chorionic villus sampling proves as reliable as amniocentesis for prenatal genetic diagnosis.

Maternal cell contamination is one possible dilemma, albeit one that can be minimized with experience. Maternal decidual tissue can be identified and excluded from preparations. The problem appears greatest in cultured preparations. Maternally derived decidua appear to undergo mitoses much more slowly than fetally derived villi, thus erroneously including maternal cells is unlikely to yield spurious results in direct preparations. In cultured preparations, maternal cells may grow and must be excluded before initiation. The one diagnostic error resulting from maternal cell contamination occurred in our early experience. We believe repetition would be rare at present.

That not all abnormal metaphases signify an abnormal infant is axiomatic in prenatal cytogenetic diagnosis. In chorionic villus sampling several special problems exist. First, relatively few cells may be available for analysis, and these cells may be derived from only a limited number of villi. Diagnostic errors caused by an abnormality restricted to a clone of cells are thus magnified. Second, clones of aneuploid or structurally abnormal cells may be especially troublesome in villi, which are not only rapidly dividing but not yet subject to the selective elimination that may occur later in pregnancy. We seek to resolve the inevitable diagnostic problems of chorionic villus sampling by initiating both direct as well as long-term (culture) preparations. Disagreements between results of direct preparations and results of cultures do arise. We thus developed and applied the following logic to determine our diagnosis:

1. Suppose one result is a male, and the other a female. The male complement is assumed to reflect fetal origin.

2. Suppose the complement is female, but results are only available from direct preparations and these are abnormal. This is considered likely to reflect fetal abnormalities. This type of preparation is less likely to show maternal contamination, especially if nonmosaic trisomy exists.

3. Suppose mosaicism occurs in both direct as well as cultured preparations. The mosaicism is then assumed truly to reflect fetal abnormalities; however, 45,X/46,XX or 45,X/46,XY mosaicism may still pose interpretive dilemmas even if there is "true" mosaicism by the operational definition.

4. Suppose mosaicism is detected only in direct preparations, but large numbers of cells (for example, 40 to 50) in cultured preparations show only a normal complement. Patients are informed of the disagreement, but the likelihood of a normal fetus is high. This approach is especially preferred if the chromosome involved is not one likely to be compatible with a live birth. Examples of "lethal" trisomies includes those for Nos. 2, 15, and 16.

5. Suppose mosaicism is found only in cultures. If possible, results are thus treated in similar fashion as that for interpretation of amniotic fluid cultures.[4] That is, true mosaicism is defined as requiring the same abnormality to be found in more than one culture or clone. Of course, if only one culture is available for chorionic villus sampling, as is often the case, the operational definition of pseudomosaicism (that is, confirmed to one culture) cannot always not be applied. In such a situation, counseling will depend on the total number of analyzable cells from direct and culture preparations, the number of growth centers, and the chromosome involved (see 4). If the abnormal complement can be demonstrated in several growth centers of one culture, true mosaicism is more likely.

Applying these and other conventional cytogenetic criteria led us to conclude that three of the six abnormalities we observed (Table II) were in fact truly reflective of fetal status. Three others probably reflected in vitro aberrations or clonal abnormalities restricted to certain villi. Many investigators attach less significance if mosaicism is confined to cultures, but we observed abnormalities apparently not reflective of fetal status in direct but not long-term cultures (case 6, Table II). Especially noteworthy was our failure to confirm

Table IV. Unbalanced translocations*

Parental abnormality	*Unbalanced segregant*	
	n	%
Robertsonian translocation		
Chorionic villus sampling	5/25	20.8
Amniocentesis	13/245	5.3
Theoretical		33
Reciprocal translocation		
Chorionic villus sampling	7/19	38.9
Amniocentesis	34/259	13.1
Theoretical		50

*European Collaborative Data, 1984, from Mikkelsen.[7]

45,X/46,XX mosaicism that fulfilled criteria established for amniotic fluid analysis, namely, presence of the abnormality in more than one flask or clone. We now believe 45,X cells to be especially common in villi, and we liberally recommend confirmatory amniocentesis whenever 45,X/46,XX or 45,X/46,XY mosaicism is detected. This same reasoning holds when we detect a trisomy for an autosome lethal in triplicate (for example, Nos. 2, 5, and 16). Criteria for interpreting confirmatory amniocentesis are those published previously,[5] irrespective of previous chorionic villus sampling results.

Even after excluding chromosomal aberrations not reflective of fetal status, the proportion of abnormalities in chorionic villus sampling will remain relatively high. Our own data are limited, but Tables IIIA and IIIB show outcome of chorionic villus sampling in a European Collaborative Series. The proportion of aneuploid fetuses in chorionic villus sampling is 4.7% between maternal ages 35 to 39, and 9.2% over age 39. The proportion of abnormalities in amniocentesis is much lower, namely, only slightly (1.5×) higher than the well-known frequencies observed in live-born infants.[6] Aneuploidy in chorionic villus sampling is also greater at 8 weeks than at 10 or 11 weeks (Table IIIB). Table IV shows frequencies of unbalanced offspring conceived by parents with a balanced translocation; frequencies are much higher in chorionic villus sampling than amniocentesis, and these frequencies approach theoretical expectations. All the above emphasize that chorionic villus sampling is occurring before selective processes that are not completely finished by time of amniocentesis.

None of the dilemmas considered above diminish our general enthusiasm for chorionic villus sampling; they merely point out that it remains investigational. Increasing experience will surely show trends that will continue to make interpretative dilemmas less common. Indeed, one must recall that pseudomosaicism occurs in 1% to 3% of amnniotic fluid analyses, but true mosaicism in only 0.1 to 0.2%.[6] Tetraploidy is now recognized as a normal phenomenon in amniotic fluid cultures, but this was once considered a dilemma. We suspect that the frequency of true mosaicism may prove more common in amniotic fluid cultures than in chorionic villus sampling, yet not so frequently as to pose insurmountable diagnostic problems. Liberal use of confirmatory amniocentesis is recommended while investigations continue to define the accuracy and reliability of chorionic villus sampling.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Federico G. Mariona, Detroit, Michigan. This report by the Northwestern Memorial Hospital group reiterates their cautious and positive approach to the use of chorionic villus sampling in first-trimester genetic diagnosis. The fact that only 188 patients have been screened in over 1 year of clinical service demonstrates a seemingly nonaggressive counseling approach. Twenty-two percent of the patients were classified ineligible for chorionic villus sampling, and 26% of the remaining patients declined having the procedure performed. We can possibly contribute their technical success and low number of procedurally related losses to this stringent patient screening program. Their careful attention to patient selection can further by evidenced by the fact that the majority of patients were sampled between 9 and 10 weeks, with no patients being included after 11 weeks' gestation. This cautious attitude contributes to a laudable policy when we consider the self-imposed anxiety and pressure of some physician groups to accumulate cases. Generally speaking, their selection and exclusion criteria can be construed as being standard.

We use a somewhat different catheter, which I no longer trim as I did during the experimental phase. The stylet is round ended and malleable and protrudes half a millimeter beyond the blunt end of a 21 or 26 cm, 1.2 mm outer-diameter Teflon catheter. This catheter is under an investigational device exemption from the Food and Drug Administration. The catheter used by the authors of this paper is twice as thick. I am curious to know what prompted their decision to select that particular catheter.

I noted that one of the diagnostic errors was related to the obtainment of an extremely small sample despite catheter size. Have you correlated the size of your samples with that of other groups using a smaller catheter or with those of your two obstetricians using different-sized catheters? My samples have gone from 0 (one case, 1.6%) to >50 mg, and one pass was sufficient in 73% of the cases.

Our standard protocol for follow-up of all sampled

patients includes a repeat ultrasound scan between 16 and 19 weeks' gestation, with concomitant obtainment of a sample of maternal serum α-fetoprotein. To date, we have not found a correlation between sample size, number of passes, and hypoechoic placental areas. Further, despite the significant increase in presample and postsample maternal serum α-fetoprotein, we still have not demonstrated a significant difference on the 16-and 19-week maternal serum α-fetoprotein reports between instrumented patients and noninstrumented patients. Have you monitored any of these parameters?

This study reports one procedurally related loss in a patient for whom two passes were needed; an adequate sample size was obtained as well as a normal complement, but she bled vaginally. Postsampling vaginal spotting has occurred in 9.6% of our patients sampled to date. Have you looked at the frequency of this event in your population?

The authors make a careful evaluation of the laboratory vagaries of their first 103 samples. I shall not attempt to verbally review their technical comments; however, I want to reference their efforts to establish formal recommendations for those involved in the cytogenetic analysis of complements from chorionic villi. Despite their stringent guidelines, they report one error in the sex determination, and six discrepancies, three of which were not confirmed.

The diagnostic disparities deserve special consideration, since 50% nonconfirmatory results justify fully the statement that chorionic villus sampling remains an investigational technique. Three of the six disparities were taken to confirmatory amniocentesis; however, two chorionic villus sampling complements were not confirmed by amniocentesis. A 45,X complement was confirmed by amniocentesis, and I am wondering, What was the parental decision? With reference to the nonconfirmatory abortion tissue complement, was this abortion spontaneous or requested on the basis of chorionic villus sampling direct and long-term cultures?

Based on the success rate of our short-term cultures, we stopped performing long-term cultures on a routine basis unless an abnormal complement was noted on the direct preparations. The latter has occurred in nine of our cases (14%).

In considering the vagaries of the cytogenetic interpretations, the authors have developed a "response protocol" for abnormal findings. This deserves emphasis and appropriate consideration, since it is only through caution and experience that this process will not increase the existing level of parental anxiety.

I am certainly interested in clinical details concerning the nine patients who opted for backup amniocentesis after a 46,XX complement was reported on chorionic villi. In our experience the perpetuation of the anxiety and emotional problems becomes a serious situation as the weeks go by.

It is clear to this reviewer that the authors' aim is to call attention to the fact that chorionic villus sampling as a first-trimester fetal diagnostic technique remains in the investigational phase. Certainly the need exists to continue assessing the risks and accuracy of chorionic villus sampling compared with that of the amniocentesis, which is the established technique.

Authors' note: Dr. Mariona has misunderstood our results. There were six abnormal complements detected. Four of these were considered reflective of the fetal complement: 45,X; 47,XY, +13; 45,X/46,XX; and 46,XX,t(13q13q) [Table I]. Three of these four were confirmed; one was not (45,X/46,XX). The other two abnormalties were suspect; consequently amniocentesis was recommended. The 45, XY,t(4;15) was in only one clone because of the extremely small amount of tissue received (0.05 mg). The other case was mosaic in direct preparations and normal in cultures, the mosaicism for a lethal chromosome, No. 15. Expectation of normal complements was confirmed at both amniocentesis and delivery.

Dr. James E. Nickel, Helena, Montana. Since the review of Rodeck[1] in 1983 in which he described the five biopsy techniques, one other technique has been added. Of these six techniques, the authors have chosen in their first paper to use the technique of guided catheter insertion with ultrasonic sector scanning. For a successful biopsy, three factors must be accomplished: yield of fetal tissue should be reliable, it should not be contaminated by maternal tissue, and there should be minimal risk to the mother and the fetus.

The complication rate of this surgery is defined as the number of patients in whom the procedures failed or was thought to cause ill effects to the patient or the pregnancy, either immediately or in the initial follow-up. In a survey of the literature of the complication, rates have run from 0% to 67%, but in larger series and after a number of cases for operator experience, the complication rate seems to be approximately 2%. After an average number of cases, approximately 70, the complication rate drops from 6% to a 2%. In the large series of cases as reported by these authors and a series of other authors and from the international studies, it is now believed that the fetal mortality rate will not be much increased over that which is reported.

One concern is the percentage of patients who bleed after this operation, which is believed to be 14%. This percentage was not reported by the authors, and we would like to ask what percentage of patients bleed following their procedures. It is thought by some authors that a number of complications occur with an increased number of repeated insertions of the catheter, these being related to bleeding, leaking of amniotic fluid, and abruptio placentae. Of major concern in this technnique is that of evaluation of sepsis. As reported in the *Chorionic Villus Biopsy Newsletter,* there are now a number of cases of sepsis with serious complications. I would ask the authors what the percentage of their infection rate is and whether they are currently doing cervical cultures.

A further concern, which is currently being answered by the authors, is that if bacterial invasion does occur at the biopsy site, is the potential of weakening of the amniotic membranes a related problem in the ongoing

series? Have they, since the initial gathering of these data, noticed any increased rate of premature rupture of the membranes?

It is noted in the paper that the risk of teratogenesis is low, since organogenesis is nearly completed by 8 weeks and their biopsies are apparenntly being done after 8 weeks. The risk of fetal malformation in this series is very low, and I wonder whether the authors have noticed, in other series in which the insertions were accomplished before 8 weeks, an increased rate of teratogenesis.

The paper of Warren,[2] in *Lancet* revealed that in approximately 49% of all cases having chorionic villus biopsy, maternal hemorrhage occurs. Do the authors use or plan on using tests such as the Kleihauer-Betke test to determine whether fetal/maternal hemorrhage has occurred?

The study by Perry[3] in 1985 reported experience similar to that of the authors, with a success rate of 93% and the only immediate complication occurring in approximately 5% of their cases. They were the first to report on patient attitudes toward this technique. It was found in their paper that an equal number of patients preferred chorionic villus biopsies as compared to amniocentesis. However, if it were known that a less than 5% complication rate occurred, then over 70% of patients were found to prefer chorionic villus biopsy. In the summary of their paper their studies revealed that chorionic villus biopsy was not universally accepted because of the 5% complication rate. I would ask the authors whether in their presurgical counseling they suggest a <5% complication rate and whether this was a major factor in the decision of 38 patients who elected not to have chorionic villus biopsy, as noted in the authors' summary table.

It was found in review of the literature at the end of 1984 that 43 centers had reported an excess of 3000 cases with a 97% success rate. The total chorionic villus biopsy fetal loss rate was 4.1%, and it was found that centers with the most experience had a fetal loss rate of 3.4%. It is suggested that the advantage of the chorionic villus biopsy is a possible reduction in the emotional and physical distress of reproduction for couples at genetic risk because of the ease of performing it in the first trimester. However, current concern is (1) that chorionic villus biopsy is more costly than amniocentesis, (2) that it takes longer to accomplish this technique, and (3) that it requires a higher level of ultrasound input. The preparation of the villi for cultures takes longer, and the direct preparations are labor intensive, requiring a much higher level of skill in the team. Have the authors currently evaluated the cost-effectiveness of this study and do the authors have suggestions as to what legal precautions we may take in freeing ourselves of the potential liability of this technique?

In their second paper the authors have explained the use of the technique of dissecting microscope and direct preparation, as well as cultured villi. This represents a shortened version of the report of Holzgreve[4] of the University of California in San Francisco in which three different methods of chromosome analysis are used. The authors have shown a marked improvement in their technique because even with use of this technique Martinville et al.[5] had reported 9% failures of cultured tissue. As can be seen from the paper the results of this technnique rely entirely upon the purity of villus tissue. In a report of possible sources of error Ridler[6] indicated that the prenatal diagnosis by means of chorionic villus sampling has not yet fulfilled its early promise. This was because of the variable success in attaining villi pure enough for culture, and because in direct preparation for karyotyping with the chorionic biopsy, the likelihood of contamination with maternal cells is much greater. It was thought by these authors that artificial mosaicism is much more common in trophoblastic tissue rather than fetal tissue, and herein may lie the possibility of the error. This point has been clearly brought up and discussed by the authors.

In the excellent summary by Blakemore[7] in 1984, with use of a modification of the Niazi culturing techniques for obtaining metaphase cells and the use of quinacrine-banded karyotypes, the results could be obtained in 5 hours from uncultured villus. The authors have not reported to us regarding the time interval for initial diagnosis. It was in the summary in Blakemore's paper that they indicated that their technique did show a high degree of reliability; however, it was felt by these authors that at the current time they were spending one and a half to two times the effort and resources used in chorionic villus biopsy compared to that for chromosomal diagnosis. I would ask the authors whether they have done a cost-analysis comparison between this technique and routine amniocentesis techniques. Do the authors feel they would have a higher failure rate currently with their techniques of villus samples that were <5 mg? Of concern to all people using this technique is the significance of mosaicism and polyploidy, which needs to be established in direct preparations and harvest of cells. It is well known that the amniocytes demonstrate a high frequency of tetraploidy, which is of no recognized significance itself, and this has been reported on numerous occasions in animal studies.

I would like to ask the authors whether they are planning an ongoing comparison study with early prenatal karyotyping done simultaneously from blood samples to establish the reliability of this technique concurrently with their technique. I would also like to ask the authors about the number of technicians required in their approach, the time required in preparations, and the length of time between the biopsy and the laboratory diagnosis.

The authors have presented a very comprehensive plan to evaluate the samples if they get questionable results on direct preparation and should be complimented on this approach. If the authors do not get enough tissue, do they eliminate the direct preparation and go directly to use of the cultures?

I would like to thank the authors for supplying me

with a summary outline of their papers before the time of the meeting and would like to compliment them for their ongoing work in what is considered at this point an alternate technique to amniocentesis. Also, I would like to ask the authors how they currently handle the liability situation, which must arise with use of this experimental technique.

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DR. ROBERT CARPENTER, Houston, Texas. One point raised by Dr. Mariona that Dr. Simpson might discuss really needs a lot of emphasis, and that is the aspect of counseling. The question was raised by him as to the number of patients who subsequently elect not to have chorionic villus sampling done. We have had 100 procedures done in our center, and at least 50% to 60% of the patients who requested the procedure did not have it done. The reasons for this are very diverse, but I think the major one is that aspect raised by Dr. Nickel, that is, what is the loss rate associated with the procedure. Couples fear that they may experience a loss due to chorionic villus sampling even when the data for an individual center show a loss rate of 2%.

In our particular center the loss rate associated with the procedure that is quoted to patients is the loss rate experienced in 9000 worldwide series of about 4%. We have had five losses in our 100 procedures. To date, those five losses have occurred from 3 days to 7 weeks after the procedure. So far we have not had preterm labor in any of the patients at more than 21 weeks' gestation.

Therefore the critical questions of loss rate and infection are also addressed and are extensively handled in our counseling sessions. The risk of infection is the one risk we cannot quantitate, and there have been relatively few cases in the 9000 procedures that have been done.

Those two particular issues, when raised, will scare many patients, and I think it is very appropriate in any counseling center dealing with chorionic villus sampling that those two issues be emphasized and emphasized again. We are probably overly counseling, and in effect, instead of being nondirective, we are being biased to a more directive conservative view.

All of the other things discussed by Dr. Elias as far as procedural techniques are concerned should be done to minimize the risk of infection.

One other question was raised concerning cervical cultures. We are doing cultures in all cases at the time of chorionic villus sampling. We currently have a continuous series of 60. We are finding a significant culture-positive rate for both mycoplasma and for *Ureaplasma,* and also we are establishing cultures from the catheter on its removal from the uterine cavity. We are also finding similar organisms as well as other organisms, including *E. coli* and group B streptococci, which did not show up on the initial culture. The meaning of that information will be seen in the future as more prospective data are collected.

DR. C. BRANDON CHENAULT San Antonio, Texas. Has consideration been given to Rh sensitization? What do you do about it as far as counseling and treatment are concerned?

DR. ELIAS (Closing). Let me begin by addressing the question of vaginal bleeding. We have followed up all of our patients very carefully and have found that about half of them had some spotting within 48 hours after the procedure. On follow-up the vast majority reflected some old blood in the vaginal vault from the site of the tenaculum grasping.

With respect to infection, we have not had any in our series to date. We routinely perform cultures for gonorrhea as a screening process, in accordance with the National Institutes of Health protocol, and we have not found any to be positive yet. We do not perform cultures for other organisms because at this time we are not sure about the significance of the inevitable cadre of organisms that one could find.

We recognize that there is a very significant potential for sepsis in these patients and we have alerted all of our physicians about this risk. We have sent out a special letter to this effect. In addition, we give our patients a handout to take home with them, which specifically discusses the warning signs and gives them telephone numbers to call in case of suspected problems. We follow up all of our patients 3 to 4 days after the procedure to find out if there have been any untoward effects.

With respect to the issue of premature rupture of the membranes, we did have one case that I discussed in the presentation. This patient had premature rupture of the membranes at 27 weeks' gestation and was delivered at 29 weeks' gestation with the infant dying of β-hemolytic streptococcal pneumonia. This is one of the major focuses of the National Institutes of Health collaborative study, and the answer to the problem of premature rupture of the membranes will hopefully be forthcoming. From personal communications with some of the other centers, we have some concerns about second-trimester rupture of the membranes associated

with oligohydramnios, and we will have to keep a very close watch on this possible association.

With respect to Rh sensitization, at the American Society of Human Genetics meeting in Salt Lake City, Utah, Dr. Karen Blakemore and her colleagues from Yale reported a study in which maternal serum α-fetoprotein levels were assessed both before and after chorionic villus sampling in 51 cases, and in 32 cases they found increases of ≥5 ng/ml. That means that in two-thirds of the cases there was some degree of fetomaternal bleeding. There was also a positive correlation with the amount of villi that were aspirated and the level of fetoprotein. We routinely administer Rh immune globulin after chorionic villus sampling to all appropriate Rh-negative candidates.

The acceptance of chorionic villus sampling is, of course, a very complex issue. We and others are currently collaborating on psychological profile studies trying to assess the various anxieties that patients undergo in making decisions between no prenatal diagnosis, chorionic villus sampling or amniocentesis. Obviously proper counseling in a neutral fashion is critical.

A question was asked concerning the cost of the procedure. We can say it is essentially the same as for a genetic amniocentesis at our institution. We do, however, add about $200 to the procedure for the ambulatory surgery suite as well as for the postoperative follow-up, which is about 45 minutes.

Legal precautions in chorionic villus sampling are germane in the context of developing a new surgical technique. We can only say that we feel it is very important to have institutional review board approval of this new procedure because, as we have tried to indicate, both the safety and the accuracy have not yet been fully delineated. We believe that before proceeding to use chorionic villus sampling in continuing pregnancies it is required to have Food and Drug Administration approval for the catheter to be used. One does not want to be caught in a situation of having a complication when the Food and Drug Administration has specifically said that only approved centers should be doing the procedure. The most important "legal precaution" is that one ought to fully adhere to the spirit as well as the letter of informed consent, and I believe that good medicine will result in good legal outcomes.

The time for results generally takes about 48 hours with direct analysis and 5 to 7 days thereafter for the cultures. Initially we found our laboratory processes to be very labor intensive, but more recently we have actually found that the time per technician is reduced because of the advantage of not having to follow cultures for prolonged periods of time. Currently we feel that a cytogenetics technician can process approximately 150 samples per year.

The question was asked whether we were doing any work with peripheral maternal blood or trophoblastic cytogenetic analysis. We have initiated a collaborative effort with Dr. Harold Miller at Michigan State University, and of course we all have hopes of developing noninvasive methods for prenatal monitoring.

If a small amount of tissue is obtained at chorionic villus sampling, as Dr. Simpson indicated, we would generally process the specimen by the culture technique because this offers a greater number of metaphases. However, each case must be individualized.

With respect to Dr. Carpenter's comment about counseling, this is obviously crucial. We would much rather have a patient decline chorionic villus sampling than have a patient agree to it who is not fully committed to the process. Our genetic counselor spends approximately 45 minutes with each couple, and we follow this up by an additional 10 to 15 minutes of discussion before the procedure.

We have also found, as others have, that the optimal time of sampling is between 9 and 11 weeks from the onset of the last menses. At less than 8 weeks one begins to have trouble documenting fetal compromise. More important, there is a very dramatic drop-off rate in the frequency of cytogenetic abnormalities after this time. In other words, if you were to sample at 6 to 7 weeks you would be ascertaining a greater number of chromosomally abnormal fetuses that would have otherwise spontaneously aborted.

Finally, in evaluating various catheters in our initial trials, we determined that samples can be more reliably obtained with the larger-sized catheter (that is, 16 gauge outer diameter).

Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women

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The possibility of Rh immune globulin failure exists when a fetomaternal hemorrhage exceeds 25 to 30 ml of whole blood and only one 300 μg vial of Rh immune globulin is administered. In this prospective study of 1000 consecutive Rh-negative women who were delivered of Rh-positive newborn infants, the presence of fetal erythrocytes in maternal blood was identified with use of both the D^u test read microscopically and the erythrocyte rosette test. All positive tests prompted fetomaternal hemorrhage quantification with use of a modified Kleihauer-Betke acid elution test. Nineteen patients demonstrated a positive rosette test, and the only positive D^u tests were in five of these 19. Six of the nineteen had levels of >30 ml of whole blood for an incidence of 0.6% for fetomaternal hemorrhage exceeding the protective capabilities of the standard Rh immune globulin dosage. In experiments with simulated fetomaternal hemorrhage, all 79 samples, containing from 2.5 to 70 ml of fetal whole blood, were positive according to the erythrocyte rosette test. Applying the D^u test to the same samples resulted in a 30% false negative rate at the level of a 30 ml simulated hemorrhage. Based on sufficient sensitivity, ease of interpretation, and reasonable cost, the rosette test appears to be a superior screening test for excessive fetomaternal hemorrhage in Rh immune globulin candidates. (Am J Obstet Gynecol 1986;154:1363-9.)

Key words: Erythrocyte rosette test, Kleihauer-Betke test, Rh immune globulin, fetomaternal hemorrhage

Administration of 300 μg of Rh immune globulin after delivery prevents Rh isoimmunization in over 98% of cases.[1-3] Despite a program of routine postpartum use of Rh immune globulin, sensitization can occur if fetomaternal hemorrhage sufficiently antedates delivery or if the amount of Rh immune globulin is inadequate to suppress the antigenic effect of a large fetomaternal hemorrhage. Bowman and Pollock[4] have shown that antepartum administration of 300 μg of Rh immune globulin at 28 weeks can reduce the incidence of the former from 1.8% to 0.07%. Nevertheless, despite use of a combination of antepartum and postpartum Rh immune globulin, cases of quantitative failure remain a possibility unless routine postpartum screening by means of a sensitive test for fetomaternal hemorrhage is used.

A 300 μg vial of Rh immune globulin contains sufficient antibody to suppress the immunogenicity of 15 ml of Rh-positive fetal red cells or 30 ml of whole blood.[5] Although the incidence of postpartum fetomaternal hemorrhage in excess of 30 ml of whole blood has been reported to be only 0.2 to 1.0%,[6-7] this subgroup of patients must be identified so that additional Rh immune globulin can be offered. Currently available tests to identify excessive fetomaternal hemorrhage include modified Kleihauer-Betke acid elution techniques, the D^u test read microscopically, erythrocyte rosette techniques, and enzyme-linked antiglobulin tests.[8] Factors such as cost, laboratory time required to complete the test, and ease in interpretation have led to use of the D^u test or rosette test during the past 5 years by a majority of laboratories where fetomaternal hemorrhage screening has been performed.[9, 10]

The purpose of this study was to screen consecutive Rh immune globulin candidates with the D^u test and the erythrocyte rosette test in parallel. In addition, both techniques were applied to mixtures of blood containing measured proportions of Rh-positive fetal cells and Rh-negative adult cells.

Material and methods

For a period fo 30 months, postpartum blood samples from 1000 consecutive Rh-negative mothers who delivered Rh-positive infants were collected in glass tubes containing ethylenediaminetetraacetate at four hospitals participating in the study. Samples were screened for fetomaternal hemorrhage at a single hospital with use of both the microscopic D^u test and the rosette test. Quantitation of the amount of fetomaternal hemor-

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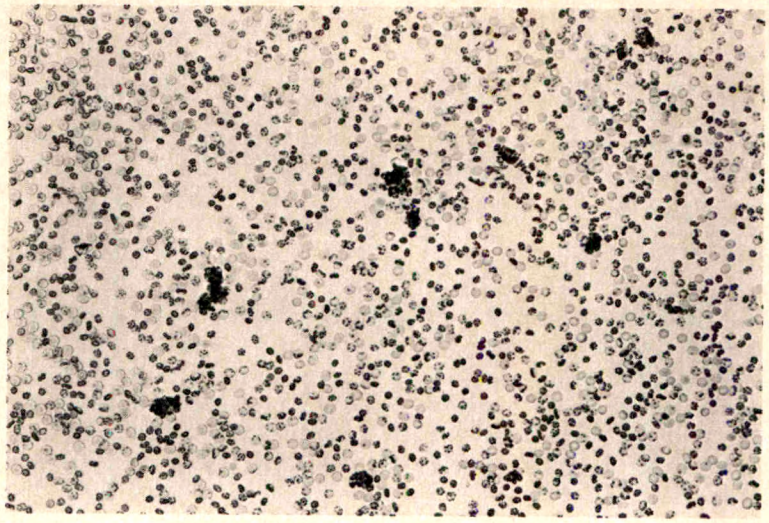


Fig. 1. Rosette test of simulated fetomaternal hemorrhage containing 0.6% fetal cells.

rhage of clinical samples that demonstrated a positive test result was determined. The method used was a modification of the Kleihauer-Betke acid elution test[11] and utilized a fetal cell staining kit (Simmler, Inc., St. Louis, Missouri). The directions of the manufacturer were followed.

In the D^u test, one drop of commercially available, chemically modified IgG anti-D was placed in a 12 × 75 mm glass tube. One drop of a once-washed 5% saline suspension of test red cells was added. The tube containing red cells and antiserum was incubated at 37° C for 15 to 20 minutes, followed by four automated saline washes to remove unbound anti-D. Two drops of commercially available, anti-IgG antiglobulin were added to the gently resuspended washed cell button. The tube was centrifuged in a tabletop centrifuge for 15 seconds at 3500 rpm. The cell button was gently resuspended, and numerous fields were examined microscopically under low power (×100). A test was interpreted as positive when agglutination was observed in any of the fields examined. The D^u test required approximately 25 minutes for completion.

In the rosette test, one drop of commercially available, chemically modified, IgG anti-D was added to a 12 × 75 mm glass tube. One drop of a once-washed 5% saline suspension of test red cells was added. The tube containing red cells and antiserum was incubated at 37° for 15 to 20 minutes, followed by four automated saline washes to remove unbound anti-D. Indicator cells were prepared by diluting commercially available, ficinized, Rh-positive (cDE) red cells. One drop of the 0.5% saline suspension of enzyme-treated Rh-positive (cDE) indicator cells was added to the resuspended washed button. The tube was immediately centrifuged in a tabletop centrifuge for 15 seconds at 3500 rpm. The cell button was resuspended, and numerous fields were examined microscopically under low power (×100). At least 10 random low-power fields were examined. A test was interpreted as positive when rosettes were present (see Fig. 1). A negative test had no rosettes and an even field of unagglutinated cells. Positive and negative controls were tested with each run of test samples. Positive controls consisted of 0.5% (CcDe) erythrocytes and 99.5% (ce) erythrocytes. Rh-negative red blood cells were used as the negative control. The rosette test required approximately 25 minutes for completion.

Group O negative (ce) adult red cells and group O positive (CcDe) cord cells were washed four times in normal saline solution. A hematocrit was performed on each sample and adjusted to be within 1% of each other. Mixtures were prepared volumetrically to yield fetal-to-maternal red cell ratios of 1.4%, 1.0%, 0.8%, 0.6%, 0.4%, 0.2%, 0.1%, 0.05%, and 0%. These mixtures were prepared to simulate blood samples from Rh-negative mothers with fetomaternal hemorrhage of 70, 50, 40, 30, 20, 10, 5, 2.5, and 0 ml whole blood, as calculated by the Kleihauer formula. These mixtures were aliquoted to prepare 100 coded samples by a technologist not directly involved in the study. One technologist (J. C. B.) tested all artificial mixtures as unknowns, using both the microscopic D^u test and the rosette test. All samples were tested within 72 hours of preparation.

The hospital records of all patients with either a positive D^u test or positive rosette test were reviewed by one of the authors for mode of delivery and complications of pregnancy.

Results

Of 1000 consecutive Rh-negative patients who were delivered of Rh-positive infants, 19 demonstrated a positive rosette test (Table I). Based on six of the 19

Table I. Testing of 19 Rh-negative patients who were delivered of Rh-positive infants

Patient No.	*D^u test*	*Rosette test*	*Quantitation by fetal cell stain* (ml)*	*Obstetric events*
1	–	+	3	Attempted external version; primary cesarean section for breech
2	–	+	4	Primary cesarean section for transverse lie; adherent placenta
3	–	+	5	No complications
4	–	+	8	No complications
5	–	+	10	Repeat cesarean section
6	–	+	10	No complications
7	–	+	13	No complications
8	–	+	13	Repeat cesarean section
9	–	+	15	Primary cesarean section for contracted pelvis
10	–	+	15	Repeat cesarean section
11	–	+	15	Repeat cesarean section
12	–	+	24	Repeat cesarean section
13	–	+	28	No complications
14	–	+	47	No complications
15	+	+	55	Heparin before birth
16	+	+	60	No complications
17	+	+	60	No complications
18	+	+	60	No complications
19	+	+	65	Primary cesarean section for breech

*Product No. 5412, Simmler, Inc., St. Louis, Missouri.

Table II. Artificial mixtures

Rh-positive fetal cells (%)	*Fetomaternal hemorrhage (ml of whole blood)*	*Positive specimens by means of D^u test*	*Positive specimens by means of rosette test*
1.4	70	9 of 10	10 of 10
1.0*	50	8 of 9	9 of 9
0.8	40	6 of 10	10 of 10
0.6	30	7 of 10	10 of 10
0.4	20	6 of 10	10 of 10
0.2	10	4 of 10	10 of 10
0.1	5	4 of 10	10 of 10
0.05	2.5	2 of 10	10 of 10
0.0	0	0 of 20	0 of 20

*One sample omitted because of contamination.

exceeding a 30 ml fetomaternal hemorrhage when quantitated with the Simmler fetal cell stain, the calculated incidence of fetomaternal hemorrhage requiring >300 μg of Rh immune globulin is 0.6%. The only positive D^u tests were in five of these 19. No sample was positive with the D^u method and negative with the rosette test. Nine of 19 (47%) were delivered by cesarean section, and 10 of 19 (53%) experienced normal labor and a vaginal delivery. Manual extraction of the placenta was performed at all nine cesarean sections.

Rosette tests on artificial mixtures containing fetal erythrocytes were positive in all 79 samples, with the calculated fetomaternal hemorrhage ranging from 2.5 to 70 ml of whole blood (Table II). One sample of 1.0% fetal cells had to be discarded because of contamination. Use of the D^u test resulted in a 30% false negative rate for a fetomaternal hemorrhage of 30 ml. At all eight concentrations of fetal cells, the rosette test was more sensitive than the D^u test. With use of a two-sided sign test for nonparametric data, the rosette test was found to be superior to the D^u test with a calculated p value of 0.0078. None of the 20 specimens containing only adult erythrocytes was positive by means of either the D^u test or rosette test.

Reagent cost added to the cost of the technologist's technical time of approximately 25 minutes yielded a laboratory cost of $6 to $7 per test for both the rosette and D^u technique. Based on 40 minutes of technical time, similar calculations produced a laboratory cost of $9 to $10 for each test by means of the Simmler fetal stain.

Comment

The presence of fetal red blood cells in the maternal circulation was first demonstrated in 1954 by Chown,[12] who agglutinated Rh-positive fetal erythrocytes in a postpartum maternal blood sample of an Rh-negative patient. In 1957 Kleihauer et al.[11] described the first test allowing differentiation of fetal erythrocytes from adult erythrocytes. They showed that at an acid pH

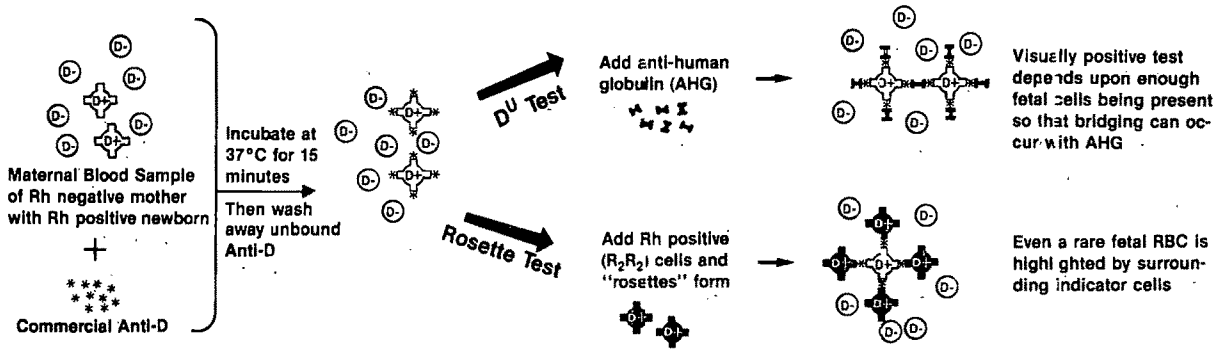


Fig. 2. Diagrammatic representation of the microscopic D^u test and erythrocyte rosette test.

level, hemoglobin A can be eluted from red blood cells whereas hemoglobin F is more resistent to elution and can be identified by special stain uptake.

Despite being the technique most frequently used in previous clinical studies of fetomaternal hemorrhage, several aspects of the Kleihauer-Betke test have discouraged routine use as a screening test. The reagents require fresh preparation, and the pH of the elution must be strictly maintained. An acid elution kit (Fetaldex, Ortho Diagnostics, Inc., Raritan, New Jersey) was introduced in 1975 as a proposed improvement over the Kleihauer-Betke method. The Fetaldex kit provided prepackaged, premeasured reagents and therefore was convenient and technically easier to use. Drawbacks to acid elution testing of all Rh negative parturients continued to include cost, time of laboratory processing, and false positive tests. Depending on the racial composition of a patient population, false positive acid elution tests can occur in 2% to 6% of individuals with hemoglobinopathies or significant elevation of hemoglobin F. Pembrey et al.[13] have reported that 6% of nonpregnant female controls manifested hemoglobin F concentrations >0.9%, a level at which all samples had positive cells on acid elution preparation.

By 1979 the most commonly used fetomaternal hemorrhage screening test was the D^u test read microscopically.[9] The term "microscopic D^u" refers to examination of a maternal blood sample with use of the antiglobulin test after incubation with incomplete anti-D typing serum (see Fig. 2). Advantages of this test include low cost as well as ease of completion in the laboratory. The major disadvantage of the D^u test is the 12% to 17% false negative rate noted in large series[10] for a simulated fetomaternal hemorrhage of 30 ml (see Table II). Our data highlight this weakness with the finding of a 30% false negative rate for a simulated 30 ml fetomaternal hemorrhage. Also of concern is the fact that the D^u test in our study yielded false negative results even for simulated fetomaternal hemorrhage >30 ml. False negative findings, of course, allow the possibility of undertreatment of the Rh immune globulin candidates.

In 1982 Sebring and Polesky[7] suggested a rapid erythrocyte rosette technique as a screening test for significant fetomaternal hemorrhage. This test differs from the D^u test at the point of the last step (see Fig. 2). Instead of adding anti-human globulin, the rosette test consists of adding enzyme-treated Rh-positive (R_2R_2) erythrocytes. Even a rare fetal erythrocyte is highlighted by surrounding indicator cells whereas the D^u test requires fetal cells to be close enough that bridging with anti-human globulin can occur. Positive rosette tests are visually striking (see Fig. 1) even to the neophyte in the laboratory. In the 1982 study of Sebring and Polesky, prepared mixtures of Rh positive fetal cells and Rh negative maternal cells were screened by means of the microscopic D^u test and rosette test. At the level of a 30 ml fetomaternal hemorrhage, the sensitivity of the rosette test was found to be 100% and the sensitivity of the D^u test to be 85%. The rosette test was recommended as the screening test of choice for fetomaternal hemorrhage.

Recently reported, fluorescence-activated cell sorter techniques[14] have suggested that fetomaternal hemorrhage at delivery is a universal event. Medearis and associates reported that the mean volume of fetal erythrocytes was 0.156 ml based on a mean Rh-positive to Rh-negative erythrocyte ratio of 1:14,100 in maternal blood. Obviously the ideal screening test would be negative for all instances of insignificant fetomaternal hemorrhage yet would be positive for all hemorrhages exceeding the protective capability of the usual postpartum dosage of Rh immune globulin.

Our findings indicate that within our population, 19 per thousand Rh immune globulin candidates have a positive rosette test and approximately 6 per thousand demonstrate fetomaternal hemorrhage in excess of 30 ml. Cost and laboratory technical hours necessitated by quantitation of only 19 tests per thousand represents minimal inconvenience for a screening test that is 100% sensitive at levels well below the critical level of a 30 ml fetomaternal hemorrhage. False positive results by means of the rosette test can occur as has already been reported by Taswell and Reisner,[15] who found a 1.5%

false positive rate among 240 blood samples containing only Rh-negative adult erythrocytes.

Because the majority of excessive fetomaternal hemorrhages cannot be predicted by obstetric events (see Table I), a screening test should be applied to the entire population at risk. Since 1981, routine screening of all Rh immune globulin candidates for excessive fetomaternal hemorrhage has been a standard of the American Association of Blood Banks[16]; subsequently, the choice of screening test rather than whether to screen has been the main subject of debate. Based on sufficient sensitivity, ease of interpretation, and reasonable cost, the erythrocyte rosette test appears to be a superior screening test for excessive fetomaternal hemorrhage in Rh immune globulin candidates. Because the rosette test demonstrates 100% sensitivity for a 10 ml fetomaternal hemorrhage in our series as well as in previous reports,[7, 15] it may also prove to be the test of choice in countries where routine postpartum treatment consists of 100 to 125 μg of Rh immune globulin.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Neil K. Kochenour, Salt Lake City, Utah. Dr. Stedman has presented a convincing case for the use of the rosette test rather than the D^u test to detect fetomaternal hemorrhage. The rosette test was sensitive enough to detect all clinically important fetomaternal hemorrhages without a large number of false positive tests.

The authors state that "the choice of screening test rather than whether to screen has been the main subject of debate." In my opinion, it is still controversial whether or not all Rh-negative women who deliver Rh-positive infants should have a test for excess fetomaternal hemorrhage. The American College of Obstetricians and Gynecologists technical bulletin No. 79, August 1984, states "Routine screening is not recommended. The volume of fetal blood entering the maternal circulation should be determined, however, for all high-risk pregnancies."[1] Although some authors have found certain obstetric manipulations to be associated with a large fetomaternal hemorrhage, the authors of this study did not find this to be true. In a study of over 1200 Rh-negative women delivering Rh-positive infants, we found no correlation between excess fetomaternal hemorrhage and any obstetric manipulation except for manual removal of the placenta.[2] Thus it is not possible to identify reliably all patients at risk by screening "high-risk" patients.

In a cost-conscious medical environment, one reason for not recommending routine screening for excess fetomaternal hemorrhage may be the question of cost-effectiveness. I would like to briefly address that issue. I recently contacted nine hospitals of varying size throughout the state of Utah. Eight of the nine hospitals routinely screened the appropriate patients. The cost to the patient varied from $11 to $27. By means of these figures the cost-effectiveness of routine screening can be estimated; by means of the least expensive cost to the patient, the cost of screening 1000 patients is $11,000. Approximately 18 patients will require a Kleihauer-Betke stain to quantify the hemorrhage: a cost of about $300. Six of these will be found to have an excess fetomaternal hemorrhage requiring extra Rh

Table I. Cost of screening for excess fetomaternal hemorrhage

Erythrocyte rosette test	
1000 patients at $11	$11,000
19 Kleihauer-Betke stains for positive test at $17	300
6 additional Rh immune globulin treatments at $60	360
	$11,360
Cost per sensitization prevented (assuming four patients sensitized)	$ 3,000
Kleihauer-Betke modification	
1000 patients at $17	$17,000
12 additional Rh immune globulin treatments $60	720
	$17,720
Cost per sensitization prevented	$ 4,000

immune globulin at a cost of $60 per dose. The total cost of the screening procedure is about $11,660. If one assumes that four of these patients would have been sensitized had the excess hemorrhage not been identified, the cost per sensitization prevented is about $3000 (see Table I).

Four of the nine hospitals I contacted use a modification of the Kleihauer-Betke stain. The least expensive charge to the patient for this type of test in the hospitals that I contacted is $17. The cost of screening 1000 women with this type of program is $17,000. Twelve additional doses of Rh immune globulin would be required. The cost per sensitization prevented with this method is more than $4000. I would point out that the costs used in these examples may vary with the number of tests performed, since some kits have reagents that are useful for a limited time. Also, the cost of the kits may vary with the quantity purchased.

We can compare these costs with the cost of caring for an isoimmunized pregnancy. Again, I have made some assumptions about the severity of the involvement, but I believe this comparison at least gives some idea of cost-effectiveness. The isoimmunized patient requires amniotic fluid analysis during her pregnancy—in this instance, four amniocenteses at $200. I have assumed a 2-day course in the newborn intensive care unit for the infant at $1000 per day, one exchange transfusion at $400, and a slightly longer maternal stay at $200 per day (Table II). This patient was not severely enough sensitized to require an intrauterine transfusion, which would have added considerably to the cost. Even with these conservative assumptions the total comes to about $3600 per pregnancy. I wish to reemphasize that the most effective way to reduce the number of Rh-isoimmunized patients is to give Rh immune globulin to all candidates post partum. Since the majority of failures are due to sensitization before delivery, the second most effective method is to use antepartum Rh immune globulin. Although the number of patients sensitized because of an excess fetomaternal hemorrhage at the time of delivery is small, the cost of screening for this is also low, about one-fifth the cost of a dose

Table II. Cost of treatment for isoimmunized patient

Ultrasound and amniocentesis, 4 at $200	$ 800
Newborn intensive care unit, 2 days at $1000	$2000
Exchange transfusion at $400	$ 400
Additional maternal hospitalization, 2 days at $200	$ 400
Cost per sensitized patient	$3600

of Rh immune globulin. I therefore feel that consideration should be given to the routine use of a screening test such as the erythrocyte rosette test.

The authors have presented a strong case for the erythrocyte rosette test to screen for excess fetomaternal hemorrhage when Rh-negative women deliver Rh-positive infants. Before one can recommend the use of this test, however, I believe that a follow-up study should be performed to determine whether any patients using this method of screening became sensitized. I wish to ask the authors whether they have any information on the follow-up of these patients and whether they plan to evaluate systematically the effectiveness of their screening program.

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Dr. Harry Farb, Minneapolis, Minnesota. Dr. Stedman and his colleagues have presented additional and encouraging information on the use of a rosette test as a more sensitive index of fetomaternal hemorrhage. In the past the available tests have included the tedious Kleihauer-Betke procedure and the relatively insensitive D^u test. The ultimate purpose is to detect excessive fetal hemorrhage into the maternal circulation at term in Rh-negative mothers and Rh-positive infants and, more recently, in the detection of fetomaternal hemorrhage in pregnant patients experiencing blunt abdominal trauma. Current Rh immune globulin prophylaxis of Rh sensitization is effective only if the fetomaternal hemorrhage is <30 ml of blood. If greater than that amount, the dose of Rh immune globulin must be increased to effectively suppress Rh sensitization. Some of this is accomplished at 28 weeks of gestation with the recently added dose of Rh immune globulin to the Rh-negative, unsensitized patient. However, even with 28-week therapy, occasional instances of excess fetomaternal hemorrhage occur, serving as a small risk of Rh sensitization.

The erythrocyte rosette test to detect fetomaternal hemorrhage is an interesting variation on D^u testing. The test is the same, the difference being that the ficin-treated Rh-positive red cells are a more sensitive index

of Rh-positive fetal cells than is antiglobulin serum. As a result, the erythrocyte rosette test will detect fetal Rh-positive red cells in maternal blood representing as little as a 2.5 ml fetomaternal hemorrhage.

A predicted result of switching from the D^u test to the erythrocyte rosette test is an increasing number of confirmatory Kleihauer-Betke tests for quantitation purposes. It also follows that more Rh immune globulin prophylaxis will be required. More philosophically and practically, the ideal test would be one that is limited to detecting only those fetomaternal hemorrhages of >30 ml of whole blood. Nevertheless, our laboratory plans to implement the erythrocyte rosette test as part of the RhoGAM workup within the next few weeks.

Of the 1000 consecutive Rh-negative patients who were delivered of Rh-positive infants in this study, 19 demonstrated a positive rosette test. Six of the 19 exceeded a 30 ml fetomaternal hemorrhage, and the only positive D^u test was in five of these six. No sample was positive by means of the D^u method when the fetomaternal hemorrhage was less than 30 ml, yet 16 of 40 artificial mixtures containing 2.5 ml to 20 ml fetal whole blood were positive by means of the D^u test. I would like to ask Dr. Stedman to comment on this finding.

Finally, I would like to point out that the D^u test failed to detect one fetomaternal hemorrhage of >30 ml among 1000 patients in this study. One might ask before replacing the D^u test with the rosette test. When is enough, enough? How completely effective does any therapeutic endeavor have to be? In addition, the American College of Obstetricians and Gynecologists has not recommended routine screening for excess fetomaternal hemorrhage, since the risk of sensitization is very low, even with hemorrhages >30 ml.[1] Would Dr. Stedman please comment on these concepts?

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Dr. Kenneth W. Crabb, St. Paul, Minnesota. First, concerning use of the rosette test at 28 weeks to determine which patients need antepartum prophylactic immunoglobulin, could we perhaps give a smaller dose, since the level of sensitivity is at 10 ml instead of 30 ml?

Second, can Dr. Stedman change the mechanical procedures of the test to change the sensitivity of the test?

Dr. Stedman (Closing). In regard to Dr. Kochenour's questions, the American College of Obstetricians and Gynecologists technical bulletin certainly conflicts with the published standards of the American Association of Blood Banks. I think it must be realized that most blood banks are now routinely screening for excessive fetomaternal hemorrhage, and we, as obstetricians, just do not know about it. If you query your local blood bank personnel, I think you will find that the majority of institutions are adhering to the standards of the American Association of Blood Banks, most commonly because it is the pathologist who determines the protocols.

The cost of screening, regardless of which test is being used, is also difficult to determine, since the blood bank usually has the charge hidden in the overall fee for Rh immunoglobulin. I think the figures that Dr. Kochenour used were very reasonable.

Concerning his question about a follow-up program, I think this is highly desirable, and we certainly hope a longitudinal study can be accomplished for those patients who did demonstrate an excessive fetomaternal hemorrhage. I might mention that one patient has demonstrated the presence of anti-D at 6 months post partum; assuming that sufficient Rh immune globulin was given after delivery, then in that particular patient one would have to theorize that the fetomaternal hemorrhage preceded delivery and anti-D was not yet detectable at the time Rh immune globulin was given.

In regard to Dr. Farb's question, I think I can best answer in this way: It is important to understand that the Kleihauer-Betke test as a gold standard is a little bit tarnished. It is just not as precise as we would like it to be. The 95% confidence interval for a value by the Kleihauer-Betke test is ±50%, and I think it is imperative to distinguish the theoretical 30 ml critical hemorrhage from the real-life situation. In fact, referring to the 1985 technical manual of the American Association of Blood Banks, administration of extra Rh immune globulin is advised even for a 20 ml hemorrhage (whole blood). Their rationale is that due to the confidence interval of the Kleihauer-Betke results, a 20 ml hemorrhage in reality might be a 30 ml hemorrhage. I think it is useful for everyone to review what is being done in their own institution.

We encountered two patients with estimated hemorrhages of 20 to 30 ml; one had a 24 ml hemorrhage and the other had a 28 ml hemorrhage. Since there were several hospitals participating in this study, we did not dictate how much Rh immune globulin was given, but both of those patients did receive two vials of Rh immune globulin.

Dr. Crabb asked a question about use of the rosette test at 28 weeks. The wonderful thing about the rosette test as a screening test is that it does not become positive for a hemorrhage that is a fraction of 1 ml; otherwise there would be so many false positives that additional technologists would be needed just to work up the positives. Therefore I think that use of the rosette test at 28 weeks would not really be helpul because sensitization can occur by exposure to <1 ml of fetal blood and the rosette test would be negative; so I really cannot endorse that application.

Dr. Crabb also asked whether the sensitivity of the test could be increased at all. I am not aware that this can be done. Again, it is solely a screening test. Currently we are performing an ongoing study evaluating the rosette test used in a quantitative fashion, and we will eventually compare the quantitative rosette test to a Kleihauer-Betke equivalent. This is for a future presentation.

Books received

Advances in International Maternal and Child Health. Volume 5. Edited by D. B. Jelliffe and E. F. Patrice Jelliffe. 188 pages, illustrated. New York, 1985, Oxford University Press. $45.00.

Antenatal Care Assessed. Marion Hall, Sally Macintyre, and Maureen Porter. 139 pages. Aberdeen, 1985, Aberdeen University Press. No price listed.

Biopsy Pathology of the Breast. J. P. Sloane. 284 pages, illustrated. New York, 1985, John Wiley & Sons, Inc. $40.00.

Clinics in Obstetrics and Gynaecology. Volume 12, Number 3. Reproductive Endocrinology. Guest Editor, Howard S. Jacobs. 220 pages, illustrated. Philadelphia, 1985, W. B. Saunders Company. $24.00.

The Elements of Graphing Data. William S. Cleveland. 323 pages, illustrated. Monterey, California, 1985, Wadsworth Advanced Books and Software. $18.95 (soft cover).

Exercise Physiology. Energy, Nutrition and Human Performance. Second Edition. William D. McArdle, Frank I. Katch, and Victor L. Katch. 696 pages, illustrated. Philadelphia, 1986, Lea & Febiger. $32.50.

Gastrointestinal and Hepatic Complications in Pregnancy. Vinod K. Rustgi and James N. Cooper. 292 pages. Somerset, New Jersey, 1986, John Wiley & Sons, Inc. No price listed.

Infection in the Female. Second Edition. William J. Ledger. 293 pages, illustrated. Philadelphia, 1986, Lea & Febiger. $51.25.

An Introduction to Medical Genetics. Eighth Edition. J. A. Fraser Roberts and Marcus E. Pembrey. 394 pages, illustrated. New York, 1985, Oxford University Press. $19.95.

Manual of Clinical Problems in Obstetrics and Gynecology. Second Edition. Edited by Michel E. Rivlin, John C. Morrison, and G. William Bates. 449 pages. Boston, 1986, Little, Brown & Company. $19.50.

Miscarriage: The Facts. Gillian C. L. Lachelin. 81 pages. New York, 1985, Oxford University Press. $12.95.

Operações Obstétricas. Albertino Da Costa Barros. 319 pages. Lisbon, Portugal, Livraria Luso-Espanhola, Lda. No price listed.

Pregnancy and Parenthood. Second Edition. Edited by Anne Lader, Eileen Hutton, and Deirdre Y. Mackay. 304 pages, illustrated. New York, 1985, Oxford University Press. $7.95.

Obstetrics in Outline. Michael D. Read and Stuart Mellor. 256 pages, illustrated. Somerset, New Jersey, 1985, John Wiley & Sons, Inc. $15.00.

What Every Pregnant Woman Should Know. Gail Sforza Brewer and Tom Brewer. 260 pages. New York, 1985, Penguin Books. $6.95.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

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Prenatal Diagnosis: Principles and Clinical Applications, July 26-31, 1986, Snowmass, Colorado. Sponsored by Department of Obstetrics and Gynecology, Texas A & M College of Medicine. For further information contact: Sharon Stermer, CME Office, Scott and White Hospital, 2401 South 31st St., Temple, TX 76508. Tel.: (817) 774-4073.

Intensive Board Review Course in Reproductive Endocrinology, September 11-14, 1986, Massachusetts General Hospital, Boston, Massachusetts. Contact: Dr. William F. Crowley, Jr., Vincent Research Laboratories, Massachusetts General Hospital, Boston, MA 02114.

Three Postgraduate Gynecology Courses: Advanced Gynecologic Oncology, September 5-7, 1986; Advances in Gynecology, September 8-10, 1986; Reproductive Endocrinology, September 11-14, 1986; Massachusetts General Hospital, Boston, Massachusetts. Contact: Har-

vard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115.

Specialty Review in Obstetrics and Gynecology: Practical Aspects, September 15-20, 1986, Chicago, Illinois. For additional information, contact the Registrar's Office. Tel.: In Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

Current Trends in Reproductive Endocrinology, Infertility and Contraception, August 9-15, 1986, Waimea Beach Hotel, in Maui, Hawaii. For information contact: Beverly J. Johnson, Program Specialist, University of Southern California, Postgraduate Medical Education, 2025 Zonal Ave., Los Angeles, CA 90033. Tel.: (213) 224-7051.

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Index to volume 154

Author index*

A

Aalders JG (*see* de Bruijn et al). 1986; **154**:1085-91
Abdella TN (*see* Sibai et al). 1986; **154**:581-90
Åberg A, Gislen L: Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant. 1986; **154**:68-74
Abrams BF, Laros RK Jr: Prepregnancy weight, weight gain, and birth weight. 1986; **154**:503-9
Acosta AA (*see* Andrews et al). 1986; **154**:848-54
Adam E (*see* Kaufman et al). 1986; **154**:1312-8
Adhate A (*see* Apuzzio et al). 1986; **154**:636-7
Ager J (*see* Michaels et al). 1986; **154**:537-46
Ahokas RA (*see* Lipshitz et al). 1986; **154**:310-4, 314-7
Aine R (*see* Lehtinen et al). 1986; **154**:158-9
Aldahl D (*see* Clark et al). 1986; **154**:490-4
Aleem FA (*see* el Tabbakh et al). 1986; **154**:892-5
Allen HH (*see* Atack et al). 1986; **154**:287-9
Almeida OD Jr, Kitay DZ: Lactation suppression and puerperal fever. 1986; **154**:940-1
Ambrose A, Cefalo RC, Bowes WA: The effects of fetal exchange transfusion with a red blood cell substitute. 1986; **154**:667-74
Ambrosecchia R (*see* Pansini et al). 1986; **154**:679-82
Anderson GD (*see* Lipshitz et al). 1986; **154**:310-4, 314-7
—— (*see* Sibai et al). 1986; **154**:581-90
Anderson J (*see* Cartwright et al). 1986; **154**:1034-8
Anderson S (*see* Herbst et al). 1985; **154**:814-22
Andersson RGG (*see* Berg et al). 1986; **154**:601-6
Andresen PJ (*see* Chacho et al). 1986; **154**:1290-9
Andrews MC, Muasher SJ, Levy DL, Jones HW Jr, Garcia JE, Rosenwaks Z, Jones GS, Acosta AA: An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries. 1986; **154**:848-54
Antenucci D (*see* Pasquali et al). 1986; **154**:139-44
Apter D (*see* Laatikainen et al). 1986; **154**:94-7
Apuzzio JJ, Unwin W, Adhate A, Nichols R: Prenatal diagnosis of fetal renal mesoblastic nephroma. 1986; **154**:636-7
—— (*see* Ganesh et al). 1986; **154**:689 (Letter), 1113-4
—— (*see* Iffy et al). 1986; **154**:623-4
Arias F, Knight AB, Tomich PB: A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes. 1986; **154**:1059-63
Artal R, Wiswell R, Romem Y, Dorey F: Pulmonary responses to exercise in pregnancy. 1986; **154**:378-83
Assali NS (*see* Rudelstorfer et al). 1986; **154**:462-70
—— (*see* Tabsh et al). 1986; **154**:411-9
Atack DB, Nisker JA, Allen HH, Tustanoff ER, Levin L: CA 125 surveillance and second-look laparotomy in ovarian carcinoma. 1986; **154**:287-9
Atrash HK, Hogue CJR, Grimes DA: Epidemiology of hydatidiform mole during early gestation. 1986; **154**:906-9
Azab I (*see* el Tabbakh et al). 1986; **154**:892-5

B

Babu A (*see* Rodriguez et al). 1986; **154**:969-70 (Letter)
Baker JR (*see* Roberts and Baker). 1986; **154**:1027-30
Bakke T (*see* Soernes and Bakke). 1986; **154**:1086-7
Balun JE (*see* Ostrea Jr et al). 1986; **154**:1014-7
Barbati A (*see* Cosmi et al). 1986; **154**:436-9
Barbieri RL, Saltzman DH, Torday JS, Randall RW, Frigoletto FD, Ryan KJ: Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers. 1986; **154**:1039-43
—— (*see* Saltzman et al). 1986; **154**:1035-8
Barela AI, Kleinman GE, Golditch IM, Menke DJ, Hogge WA, Golbus MS: Septic shock with renal failure after chorionic villus sampling. 1986; **154**:1100-2
Barnea ER (*see* Oelsner et al). 1986; **154**:569-72
Barnes RB, Cha K-Y, Lee DG, Lobo RA: Modulation of luteinizing hormone immunoreactivity and bioactivity by dopamine but not norepinephrine in women. 1986; **154**:445-50
Baron JA: Cigarette smoking and endometrial cancer. 1986; **154**:965 (Letter)
Barr R (*see* Jarrell et al). 1986; **154**:306-9
Barradas MA (*see* Jeremy et al). 1986; **154**:212-3 (Letter)
Barrat J (*see* Erny et al). 1986; **154**:525-9
Barron WM, Mujais SK, Zinaman M, Bravo EL, Lindheimer MD: Plasma catecholamine responses to physiologic stimuli in normal human pregnancy. 1986; **154**:80-4
Bartlett KH (*see* Chow et al). 1986; **154**:120-6
Bassi P (*see* Pansini et al). 1986; **154**:679-82
Bastide A, Manning F, Harman C, Lange I, Morrison I: Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios. 1986; **154**:895-900
Bates SE (*see* Reece et al). 1986; **154**:150-1
Battaglia FC (*see* Johnson et al). 1986; **154**:1146-51
Baudin JC (*see* Stedman et al). 1986; **154**:1363-9
Baum LD (*see* Elias et al). 1986; **154**:1349-52
Baumann H (*see* Huch et al). 1986; **154**:996-1000
Bedard MP (*see* Shankaran et al). 1986; **154**:53-7
Beeson CC (*see* Luber et al). 1986; **154**:1264-70
Bejamin A (*see* Bodmer et al). 1986; **154**:244-50
Belaisch-Allart J (*see* Frydman et al). 1986; **154**:550-5
Belbeck L (*see* Jarrell et al). 1986; **154**:306-9
Bell D, Johansson D, McLean FH, Usher RH: Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? 1986; **154**:235-9
Bell T (*see* Kiviat et al). 1986; **154**:865-73
Benoit J, Merrill S, Rundell C, Meeker CI: Amniostat-FLM: An initial clinical trial with both vaginal pool and amniocentesis samples. 1986; **154**:65-8

*January, 1-220; February, 221-476; March, 477-698; April, 699-972; May, 973-1172; June, 1173-1438.

Benveniste R (*see* Moise et al). 1986; **154**:1080-5
Benzie RJ (*see* Garden et al). 1986; **154**:221-5
Berg G, Andersson RGG, Ryden G: α-Adrenergic receptors in human myometrium during pregnancy. 1986; **154**: 601-6
Bergamini C (*see* Pansini et al). 1986; **154**:679-82
Bergen B (*see* Ganesh et al). 1986; **154**:1113-4
Bergman BB (*see* Larsson and Bergman). 1986; **154**:107-8
—— (*see* Larsson et al). 1986; **154**:1167-8 (Letter)
Berkowitz RL, Chitkara U, Goldberg JD, Wilkins I, Chervenak FA: Intravascular transfusion in utero: The percutaneous approach. 1986; **154**:622-3
—— (*see* Goldberg et al). 1986; **154**:546-50
Bhatia R, Koppitch FC, Sokol RJ, Evans MI: Improving the yield of direct chorionic villus slide preparations. 1986; **154**:408-11
—— (*see* Shankaran et al). 1986; **154**:53-7
Birkenfeld A (*see* Ohel et al). 1986; **154**:619-21
Bissonnette JM (*see* Veille et al). 1986; **154**:1160-3
Bixler C (*see* Hoff and Bixler). 1986; **154**:126-9
Blakemore KJ (*see* Sarkar et al). 1986; **154**:390-3
Blanco JD (*see* Patterson et al). 1986; **154**:1111-2
Block SM (*see* Johnson et al). 1986; **154**:1146-51
Blumenfeld Z, Kuhn RW, Jaffe RB: Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion. 1986; **154**:606-12
Bocking AD, Harding R: Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep. 1986; **154**:655-62
——, ——, Wickham PJD: Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep. 1986;329-35
Bodmer B, Benjamin A, McLean FH, Usher RH: Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? 1986; **154**:244-50
Boler LR Jr (*see* Gleicher et al). 1986; **154**:1044-9
Bolton RN (*see* Prins et al). 1986; **154**:1275-9
Bombard A (*see* Elias et al). 1986; **154**:1349-52
Borenstein R (*see* Kletzky et al). 1986; **154**:431-5
Boros SJ (*see* Landrum et al). 1986; **154**:1114-5
Borten M, Friedman EA: Amniotic fluid ferning in early gestation. 1986; **154**:628-30
Bottoms SF (*see* Hassan et al). 1986; **154**:428-30
Boucher M (*see* Patkos et al). 1986; **154**:1107-8
Boulter G (*see* Jarrell et al). 1986; **154**:231-4
Bourgeois FJ, Thiagarajah S, Harbert GM Jr, DiFazio C: Profound hypotension complicating magnesium therapy. 1986; **154**:919-20
Bowen LW, Sand PK, Ostergard DR: Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum. 1986; **154**:145-6
Bowes WA (*see* Ambrose et al). 1986; **154**:667-74
Bowes WA Jr (*see* Seeds and Bowes Jr). 1986; **154**:1105-7
Boyers SP (*see* Oelsner et al). 1986; **154**:569-72
Bracero L (*see* Fleischer et al). 1986; **154**:806-13
Brach B (*see* Cartwright et al). 1986; **154**:1064-8
Bracken MB (*see* Srisuphan and Bracken). 1986; **154**:14-20
Braddock SW (*see* Frederick et al). 1986; **154**:1116-7
Braithwaite NDJ, Milligan JE, Shennan AT: Fetal heart rate monitoring and neonatal mortality in the very preterm infant. 1986; **154**:250-4
Bravo EL (*see* Barron et al). 1986; **154**:80-4
Brewer JI: Change and consistency. 1986; **154**:1-2 (Editorial)
Brinkman CR III (*see* Rudelstorfer et al). 1986; **154**:462-70
Brooke M (*see* Cartwright et al). 1986; **154**:1064-8
Broussard PM (*see* Patkos et al). 1986; **154**:1107-8
Brown D (*see* Cartwright et al). 1986; **154**:1064-8
Brown MA, Gallery EDM: Sodium excretion in human pregnancy: A role for arginine vasopressin. 1986; **154**:914-9
Broyles K (*see* Lipshitz et al). 1986; **154**:310-4
Brumback CL (*see* Maleck et al). 1986; **154**:625-6
Brunham RC (*see* Paavonen et al). 1986; **154**:556-64
Buck HW (*see* Quadagno et al). 1986; **154**:1018-23
Buckley J (*see* Powell et al). 1986; **154**:565-9
Bucovaz ET (*see* Morrison et al). 1986; **154** 900-3
—— (*see* Page et al). 1986; **154**:1306-11
Bulletti C, Jasonni VM, Lubicz S, Flamigni C, Gurpide E: Extracorporeal perfusion of the human uterus. 1986; **154**: 683-8
Burgio KL, Robinson JC, Engel BT: The role of biofeedback in Kegel exercise training for stress urinary incontinence. 1986; **154**:58-64
Burk RD, Kadish AS, Calderin S, Romney SL: Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear. 1986; **154**:982-9
Burry K (*see* Reynolds et al). 1986; **154**:74-9
Butterfield AB (*see* King et al). 1986; **154**:323-30
Byrd JR (*see* McDonough et al). 1986; **154**:737-48

C

Cadesky K (*see* Doran et al). 1986; **154**:277-81
Caine ME, Mueller-Heubach E: Kell sensitization in pregnancy. 1986; **154**:85-90
Calderin S (*see* Burk et al). 1986; **154**:982-9
Calkoen-Carpay TvB (*see* de Bruijn et al). 1986; **154**:1088-91
Callegari PB (*see* Ho Yuen and Callegari). 1986; **154**:273-6
Callen PW (*see* Hashimoto et al). 1986; **154**:1069-72
Calway-Fagan N (*see* Cartwright et al). 1986; **154**:1064-8
Camiel MR: Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon. 1986; **154**:1104-5
Campbell WA (*see* Feinstein et al). 1986; **154**:159-60
Campeau JD (*see* Ono et al). 1986; **154**:709-16
Cantrell CJ (*see* Paul et al) 1986; **154**:1187-93
Capello T (*see* Doran et al). 1986; **154**:277-81
Cappinarri D (*see* Cartwright et al). 1986; **154**:1064-8
Caramelli K (*see* Notelovitz et al). 1986; **154**:1009-13
Carlson CV (*see* Reynolds et al). 1986; **154**:74-9
Carmichael JA, Clarke DH, Moher D, Ohlke ID, Karchmar EJ: Cervical carcinoma in women aged 34 and younger. 1986; **154**:264-9
—— (*see* Deigan et al). 1986; **154**:255-9
Carpenter MW (*see* Coustan et al) 1986; **154**:1031-5
Carpiniello V (*see* Sedlacek et al). 1986; **154**:494-6
Carr BR, Mason JI, Mitchell MD: Prostaglandin secretion by adrenal tissue of human anencephalic fetuses. 1986; **154**:373-8
Carter D (*see* Sarkar et al). 1986; **154**:390-3
Carter V: Another technique for resolution of shoulder dystocia. 1986; **154**:964 (Letter)
Cartwright PS, Raynor D, Anderson J, Tuttle D, Moore R, Brach B, Parks R, Cappinarri D, Brown D, Calway-Fagan N, Swafford M, Etherington C, Hartlyn A, Taninecz A, Houseman M, Brooke M, Merry J: Reported sexual assault in Nashville–Davidson County, Tennessee, 1980 to 1982. 1986; **154**:1064-8
Casper RF, Lye SJ: Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep. 1986; **154**:301-5
Cassell GH (*see* Gibbs et al). 1986; **154**:717-26
Castaldo TW (*see* Koerten et al). 1986; **154**:526-8
Castellano C (*see* Lancaster et al). 1986; **154**:115-9

Castillo R (*see* Devoe et al). 1986; **154**:931-6
Caufriez A: Male pseudohermaphroditism due to 17-ketoreductase deficiency: Report of a case without gynecomastia and without vaginal pouch. 1986; **154**:148-9
Cefalo RC (*see* Ambrose et al). 1986; **154**:667-74
Cepeda EE (*see* Shankaran et al). 1986; **154**:53-7
Cha K-Y (*see* Barnes et al). 1986; **154**:445-50
Chacho KJ, Chacho MS, Andresen PJ, Scommegna A: Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay. 1986; **154**:1290-9
Chacho MS (*see* Chacho et al). 1986; **154**:1290-9
Chambers CB, Unis JS: Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus. 1986; **154**:1194-9
Chang RJ (*see* Steingold et al). 1986; **154**:1241-8
Chase JS (*see* Check and Chase). 1986; **154**:155-6
Check JH, Chase JS: Ovarian pregnancy with a contralateral corpus luteum: Case report. 1986; **154**:155-6
Chen YM (*see* Ott et al). 1986; **154**:690 (Letter)
Chervenak FA (*see* Berkowitz et al). 1986; **154**:622-3
Chitkara U (*see* Berkowitz et al). 1986; **154**:622-3
Chow AW, Percival-Smith R, Bartlett KH, Goldring AM, Morrison BJ: Vaginal colonization with *Escherichia coli* in healthy women. 1986; **154**:120-6
Clark KE (*see* Miodovnik et al). 1986; **154**:394-401
Clark SL, Greenspoon JS, Aldahl D, Phelan JP: Severe preeclampsia with persistent oliguria: Management of hemodynamic subsets. 1986; **154**:490-4
——, Pavlova Z, Greenspoon J, Horenstein J, Phelan JP: Squamous cells in the maternal pulmonary circulation. 1986; **154**:104-6
Clarke DH (*see* Carmichael et al). 1986; **154**:264-9
Cohen-Addad N (*see* Iffy et al). 1986; **154**:623-4
Collins J (*see* Jarrell et al). 1986; **154**:231-4
Cook C-J (*see* Shime et al). 1986; **154**:341-5
Cooper ES (*see* Stedman et al). 1986; **154**:1363-9
Copel JA, Pilu G, Kleinman CS: Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography. 1986; **154**:1121-32 (Curr. dev.)
Corey L (*see* Paavonen et al). 1986; **154**:556-64
Cosmi EV, Saitto C, Barbati A, Del Bolgia F, Di Renzo GC, Grossmann G, Lachmann B, Robertson B: Effect of aminophylline on lung maturation in preterm rabbit fetuses. 1986; **154**:436-9
Costerton JW (*see* Jacques et al). 1986; **154**:648-55
Cotton DB, Jones MM, Longmire S, Dorman KF, Tessem J, Joyce TH III: Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema. 1986; **154**:91-3
——, Longmire S, Jones MM, Dorman KF, Tessem J, Joyce TH III: Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion. 1986; **154**:1053-9
—— (*see* Hobson et al). 1986; **154**:1023-6
Councell RB (*see* Solano Jr and Councell). 1986; **154**:1117-8
Coustan DR, Widness JA, Carpenter MW, Rotondo L, Pratt DC, Oh W: Should a fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? 1986; **154**:1031-5
—— (*see* Cowett et al). 1986; **154**:1098-100
—— (*see* Reece et al). 1986; **154**:150-1
Cowett RM, Coustan DR, Oh W: Effects of maternal transport on admission patterns at a tertiary care center. 1986; **154**:1098-100
Cox K (*see* Strickland et al). 1986; **154**:146-8
Creasy RK (*see* Gonik and Creasy). 1986; **154**:3-8 (Clin. opinion)
Critchlow CW (*see* Paavonen et al). 1986; **154**:556-64
Cunnane M (*see* Sedlacek et al). 1986; **154**:494-6

D

Dacus JV (*see* Taslimi et al). 1986; **154**:1327-32
D'Alton M, Romero R, Grannum P, DePalma L, Jeanty P, Hobbins JC: Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease. 1986; **154**:532-7
Damien N (*see* Sachs et al). 1986; **154**:156-7
Dandona P (*see* Jeremy et al). 1986; **154**:212-3 (Letter)
Dattel BJ, Lam F, Roberts JM: Failure to demonstrate decreased β-adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition. 1986; **154**:450-6
Davidson BJ (*see* Wang-Cheng and Davidson). 1986; **154**:924-5
Davis H (*see* Devoe et al). 1986; **154**:931-6
Davis JK (*see* Gibbs et al). 1986; **154**:717-26
Dawood MY, Khan-Dawood FS: Human ovarian oxytocin: Its source and relationship to steroid hormones. 1986; **154**:756-63
—— (*see* Snowden et al). 1986; **154**:440-4
Daya S (*see* Jarrell et al). 1986; **154**:231-4
de Bruijn HWA, Calkoen-Carpay TvB, Jager S, Duk JM, Aalders JG, Fleuren GJ: The tumor marker CA 125 is a common constituent of normal cervical mucus. 1986; **154**:1088-91
Deaver JE, Leppert PC, Zaroulis CG: Neonatal alloimmune thrombocytopenic purpura: A case report. 1986; **154**:153-5
DeCherney AH (*see* Oelsner et al). 1986; **154**:569-72
Defoort P, Thiery M: A sonographic sign for fetal Down's syndrome. 1986; **154**:1169-70 (Letter)
Deigan EA, Carmichael JA, Ohlke ID, Karchmar J: Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases. 1986; **154**:255-9
Del Bolgia F (*see* Cosmi et al). 1986; **154**:436-9
Del Granado A (*see* Gleicher et al). 1986; **154**:1044-9
D'Eletto R (*see* Weiss et al). 1986; **154**:749-55
Delgado G (*see* Lancaster et al). 1986; **154**:115-9
Denney NW (*see* Quadagno et al). 1986; **154**:1018-23
DePalma L (*see* D'Alton et al). 1986; **154**:532-7
Der Yuen D: A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance. 1986; **154**:1206-10
DeRouen T (*see* Paavonen et al). 1986; **154**:556-64
Devereaux DL (*see* Ono et al). 1986; **154**:709-16
DeVinna R (*see* Ono et al). 1986; **154**:709-16
Devoe LD, Castillo R, McKenzie J, Searle N, Robinson B, Davis H: Sequential nonstress testing with use of each fetus as its own control. 1986; **154**:931-6
Di Renzo GC (*see* Cosmi et al). 1986; **154**:436-9
Diebel ND (*see* Morales et al). 1986; **154**:591-5
Dierker LJ Jr, Rosen MG, Thompson K, Lynn P: Midforceps deliveries: Long-term outcome of infants. 1986; **154**:764-8
DiFazio C (*see* Bourgeois et al). 1986; **154**:919-20
Dillard EA Jr (*see* Moseley et al). 1986; **154**:992-5
Dinh TV (*see* Moseley et al). 1986; **154**:992-5
—— (*see* Nagamani et al). 1986; **154**:384-9
Dispenziere B (*see* Ganesh et al). 1986; **154**:1113-4
Dixon LA (*see* Quadagno et al). 1986; **154**:1018-23
diZerega GS (*see* Ono et al). 1986; **154**:709-16
Dombrowski MP (*see* Hassan et al). 1986; **154**:428-30

Donnenfeld AE, Roberts NS, Losure TA, Mellen AW: Perforated adenocarcinoma of the appendix during pregnancy. 1986; **154**:637-8
Doran TA, Cadesky K, Wong PY, Mastrogiacomo C, Capello T: Maternal serum α-fetoprotein and fetal autosomal trisomies. 1986; **154**:277-81
Dorey F (*see* Artal et al). 1986; **154**:378-83
Dorko ME, Hayashi TT: Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy. 1986; **154**:801-5
Dorman KF (*see* Cotton et al). 1986; **154**:91-3, 1053-9
Dougherty M (*see* Notelovitz et al). 1986; **154**:1009-13
Dreyer NA (*see* Lanes et al). 1986; **154**:989-91
Druzin ML, Hutson JM, Edersheim TG: Relationship of baseline fetal heart rate to gestational age and fetal sex. 1986; **154**:1102-3
Duda M (*see* Sueldo et al). 1986; **154**:424-7
Duff P (*see* Schipul Jr et al). 1986; **154**:967 (Letter)
Duk JM (*see* de Bruijn et al). 1986; **154**:1088-91
Dunetz C (*see* Hart et al). 1986; **154**:878-83
Durack DT (*see* Seaworth and Durack). 1986; **154**:180-8 (Curr. dev.)

E

Edersheim TG (*see* Druzin et al). 1986; **154**:1102-3
Egan JFX (*see* Reece et al). 1986; **154**:150-1
Egley CC: Severe hypoglycemia associated with the HELLP syndrome. 1986; **154**:211-2 (Letter reply)
el Tabbakh GH, Lotfy I, Azab I, Rahman HA, Southren AL, Aleem FA: Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings. 1986; **154**:892-5
Elias S, Simpson JL, Martin AO, Sabbagha R, Bombard A, Rosinsky BJ, Baum LD: Chorionic villus sampling in continuing pregnancies. I. Low fetal loss rates in initial 109 cases. 1986; **154**:1349-52
—— (*see* Martin et al). 1986; **154**:1353-62
Ellison PT, Lager C: Moderate recreational running is associated with lowered salivary progesterone profiles in women. 1986; **154**:1000-3
Engel BT (*see* Burgio et al). 1986; **154**:58-64
Erny R, Pigne A, Prouvost C, Gamerre M, Malet C, Serment H, Barrat J: The effects of oral administration of progesterone for premature labor. 1986; **154**:525-9
Erskine RLA, Ritchie JWK: The effect of maternal consumption of alcohol on human umbilical artery blood flow. 1986; **154**:318-21
——, ——, Zaltz A, Tice T: Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death. 1986; **154**:1109-10
Eschenbach DA (*see* Kiviat et al). 1986; **154**:865-73
—— (*see* Paavonen et al). 1986; **154**:556-64
Etherington C (*see* Cartwright et al). 1986; **154**:1064-8
Evans MI (*see* Bhatia et al). 1986; **154**:408-11
—— (*see* Hassan et al). 1986; **154**:428-30

F

Fabbri R (*see* Pasquali et al). 1986; **154**:139-44
Fainstat T (*see* Hensleigh et al). 1986; **154**:978-81 (Clin. opinion)
Falcone T, Ferenczy A: Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods. 1986; **154**:260-4
Fallenstein F (*see* Huch et al). 1986; **154**:996-1000
Farmakides G (*see* Fleischer et al). 1986; **154**:806-13
Feinstein SJ, Lodeiro JG, Vintzileos AM, Campbell WA, Montgomery JT, Nochimson DJ: Sinusoidal fetal heart rate pattern after administration of nalbuphine hydrochloride: A case report. 1986; **154**:159-60
Felice ME (*see* Long et al). 1986; **154**:1164 (Letter reply)
Ferenczy A (*see* Falcone and Ferenczy). 1986; **154**:260-4
Ferrara B (*see* Landrum et al). 1986; **154**:1114-5
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Randall RW (*see* Barbieri et al). 1986; **154**:1039-43
Rankin JHG (*see* Landauer et al). 1986; **154**:1152-5
Rasmussen N (*see* Hegedüs et al). 1986; **154**:1164 (Letter)
Rattan PK, Knuppel RA, O'Brien WF, Scerbo JC: Cesarean delivery of the second twin after vaginal delivery of the first twin. 1986; **154**:936-9
Ray DA, Yeast JD, Freeman RK: The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman. 1986; **154**:1257-63
Raynor D (*see* Cartwright et al). 1986; **154**:1064-8
Reece EA, Egan JFX, Coustan DR, Tamborlane W, Bates SE, O'Neill TM, Fitzpatrick JG: Coronary artery disease in diabetic pregnancies. 1986; **154**:150-1
—— (*see* Pilu et al). 1986; **154**:630-2
Reeder SR: Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery. 1986; **154**:487-9
Reid RL (*see* Maddocks et al). 1986; **154**:573-81
Reindollar RH (*see* McDonough et al). 1986; **154**:737-48
Rekrut KA (*see* Warford et al). 1986; **154**:21-8
Reller LB (*see* McGregor et al). 1986; **154**:98-103
Resnik R (*see* Moore et al). 1986; **154**:29-33
Reynolds JW, Burry K, Carlson CV: Fetoplacental steroid metabolism in prolonged pregnancies. 1986; **154**:74-9
Reynolds SL (*see* Lipshitz et al). 1986; **154**:314-7
Ries LG (*see* Yancik et al). 1986; **154**:639-47 (Curr. Dev.)
Ritchie JWK (*see* Erskine and Ritchie). 1986; **154**:318-21
—— (*see* Erskine et al). 1986; **154**:1109-10
Ritter M (*see* Poindexter III et al). 1986; **154**:946-50 (Symposium)
Roberts AB, Baker JR: Serum fructosamine: A screening test for diabetes in pregnancy. 1986; **154**:1027-30
Roberts JM, Lewis V, Mize N, Tsuchiya A, Starr J: Human platelet α-adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit. 1986; **154**:206-10
—— (*see* Dattel et al). 1986; **154**:450-6
Roberts NS (*see* Donnenfeld et al). 1986; **154**:637-8
Robertson B (*see* Cosmi et al). 1986; **154**:436-9
Robertson CL: Disagreement on use of water-soluble contrast material in small bowel obstruction. 1986; **154**:690-1 (Letter reply)
Robinson B (*see* Devoe et al). 1986; **154**:931-6
Robinson JC (*see* Burgio et al). 1986; **154**:58-64
Rochelson B (*see* Fleischer et al). 1986; **154**:806-13
Rodriguez JG, Babu A, Verma RS: A rapid method of culturing bloody amniotic fluid for chromosome analysis. 1986; **154**:969-70 (Letter)
Romein L (*see* Lazarchick et al). 1986; **154**:1050-2
Romem Y (*see* Artal et al). 1986; **154**:378-83
Romero R (*see* D'Alton et al). 1986; **154**:532-7
—— (*see* Hobbins et al). 1986; **154**:215 (Letter reply)
—— (*see* Pilu et al). 1986; **154**:630-2

Romney SL (*see* Burk et al). 1986; **154**:982-9
Rose JC (*see* Greiss Jr et al). 1986; **154**:831-8
Rosen MG (*see* Dierker Jr et al). 1986; **154**:764-8
Rosenfeld CR (*see* Perales et al). 1986; **154**:195-203
—— (*see* Strickland et al). 1986; **154**:146-8
Rosenthal DM (*see* Spivak et al). 1986; **154**:355-61
Rosenwaks Z (*see* Andrews et al). 1986; **154**:848-54
Rosinsky BJ (*see* Elias et al). 1986; **154**:1349-52
—— (*see* Martin et al). 1986; **154**:1353-62
Ross RK (*see* Henderson et al). 1986; **154**:1181-6
Rotondo L (*see* Coustan et al) 1986; **154**:1031-5
Roy S (*see* Gibbons et al). 1986; **154**:456-61
Rudelstorfer R, Tabsh K, Khoury A, Nuwayhid B, Brinkman CR III, Assali NS: Heat flux and oxygen consumption of the pregnant uterus. 1986; **154**:462-70
—— (*see* Tabsh et al). 1986; **154**:411-9
Rundell C (*see* Benoit et al). 1986; **154**:65-8
Rurak DW, Taylor SM: Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital. 1986; **154**:674-8
Ryan KJ (*see* Barbieri et al). 1986; **154**:1039-43
Ryden G (*see* Berg et al). 1986; **154**:601-6

S

Sabbagha R (*see* Elias et al). 1986; **154**:1349-52
Sachs BP, Lorell BH, Mehrez M, Damien N: Constrictive pericarditis and pregnancy. 1986; **154**:156-7
Sacks DA: Percutaneous umbilical sampling in immune thrombocytopenic purpura. 1986; **154**:214-5 (Letter)
——, Koppes RH: Blood transfusion and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology. 1986; **154**:483-6 (Clin. opinion)
Sadovsky E (*see* Ohel et al). 1986; **154**:619-21
St. Clair PJ (*see* Gibbs et al). 1986; **154**:717-26
Saitto C (*see* Cosmi et al). 1986; **154**:436-9
Sakamoto H, Huszar G: Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta. 1986; **154**:402-7
Saltzman DH, Barbieri RL, Frigoletto FD Jr: Decreased fetal cord prolactin concentration in diabetic pregnancies. 1986; **154**:1035-8
—— (*see* Barbieri et al). 1986; **154**:1039-43
Sama JC (*see* Ganesh et al). 1986; **154**:689 (Letter)
Sánchez RA, Glenny JE, Marcó E, Voto LS, Lapidus AM, Iglesias GH, Moledo LV, Margulies M: Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women. 1986; **154**:910-3
Sand PK (*see* Bowen et al). 1986; **154**:145-6
—— (*see* Ory et al). 1986; **154**:1299-1306
Sandvei R (*see* Steier et al). 1986; **154**:1091-4
Sanfilippo JS, Wakim NG, Schikler KN, Yussman MA: Endometriosis in association with uterine anomaly. 1986; **154**:39-43
Santos C (*see* Lancaster et al). 1986; **154**:115-9
Sarkar S, Kacinski BM, Kohorn EI, Merino MJ, Carter D, Blakemore KJ: Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line. 1986; **154**:390-3
Sauerbrei EE (*see* Low et al). 1986; **154**:345-51
Scerbo JC (*see* Rattan et al). 1986; **154**:936-9
Schenken RS, Werlin LB, Williams RF, Prihoda TJ, Hodgen GD: Histologic and hormonal documentation of the luteinized unruptured follicle syndrome. 1986; **154**:839-47
Schikler KN (*see* Sanfilippo et al). 1986; **154**:39-43
Schipul AH Jr, Kopelman JN, Duff P: Maternal and fetal surveillance for older pregnant women. 1986; **154**:967 (Letter)
Schmidt GE, Kim MH, Mansour R, Torello L, Friedman CI: The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo. 1986; **154**:727-36
Schneider KTM (*see* Huch et al). 1986; **154**:996-1000
Schoenfeld C (*see* Weiss et al). 1986; **154**:749-55
Schonberg SA (*see* Hogge et al). 1986; **154**:1249-52
Schray MF (*see* Podratz et al). 1986; **154**:1319-26
Schulman H (*see* Fleischer et al). 1986; **154**:806-13
Schwartz AL (*see* Notelovitz et al). 1986; **154**:1009-13
Schwartz ME (*see* Hunter et al). 1986; **154**:633-4
Scommegna A (*see* Chacho et al). 1986; **154**:1290-9
—— (*see* Moise et al). 1986; **154**:1080-5
Searle N (*see* Devoe et al). 1986; **154**:931-6
Seaworth BJ, Durack DT: Infective endocarditis in obstetric and gynecologic practice. 1986; **154**:180-8 (Curr. dev.)
Sedlacek TV, Cunnane M, Carpiniello V: Colposcopy in the diagnosis of penile condyloma. 1986; **154**:494-6
Seeds JW, Bowes WA Jr: Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization. 1986; **154**:1105-7
Seitchik J: Words, thoughts, and things. 1986; **154**:699-708 (Pres. address)
Sell A (*see* Mogensen et al). 1986; **154**:1165-6 (Letter)
Senior CC, Steigrad SJ: Are preoperative antibiotics helpful in abdominal hysterectomy? 1986; **154**:1004-8
Seppälä M (*see* Julkunen et al). 1986; **154**:1076-9
Serment H (*see* Erny et al). 1986; **154**:525-9
Sgarlata CS, Hertelendy F: Prostaglandins and endometriosis. 1986; **154**:964 (Letter)
Shangold GA (*see* Kletzky and Shangold). 1986; **154**:362-7
Shankaran S, Cepeda EE, Ilagan N, Maricna F, Hassan M, Bhatia R, Ostrea E, Bedard MP, Poland RL: Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage. 1986; **154**:53-7
Shennan AT (*see* Braithwaite et al). 1986; **154**:250-4
Shime J, Gresser CD, Rakowski H: Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth. 1986; **154**:294-300
——, Librach CL, Gare DJ, Cook C-J: The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study. 1986; **154**:341-5
Shin DH (*see* Ho Yuen et al). 1986; **154**:336-40
Sibai BM, Abdella TN, Spinnato JA, Anderson GD: Eclampsia. V. The incidence of nonpreventable eclampsia. 1986; **154**:581-90
—— (*see* Mabie and Sibai). 1986; **154**:211 (Letter)
—— (*see* Taslimi et al). 1986; **154**:1327-32
Siitonen A (*see* Haukkamaa et al). 1986; **154**:520-4
Silbar EL: Factors related to the increasing cesarean section rates for cephalopelvic disproportion. 1986; **154**:1095-8
Simpson JL (*see* Elias et al). 1986; **154**:1349-52
—— (*see* Martin et al). 1986; **154**:1353-62
Sipos L (*see* Paul et al) 1986; **154**:1187-93
Skillman CA (*see* Miodovnik et al). 1986; **154**:394-401
Snowden EU, Khan-Dawood FS, Dawood MY: Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives. 1986; **154**:440-4
Soderstrom RM: Sterilization failures. 1986; **154**:970 (Letter reply)
Soernes T, Bakke T: The length of the human umbilical cord in vertex and breech presentations. 1986; **154**:1086-7
Sokol RJ (*see* Bhatia et al). 1986; **154**:408-11

Solano FX Jr, Councell RB: Folate deficiency presenting as pancytopenia in pregnancy. 1986; **154**:1117-8
Southren AL (*see* el Tabbakh et al). 1986; **154**:892-5
Speck G: Correct use of Hegar's sign. 1986; **154**:691 (Letter)
Spencer R (*see* Hensleigh et al). 1986; **154**:978-81 (Clin. opinion)
Spillman T (*see* Hobson et al). 1986; **154**:1023-6
Spinnato JA (*see* Sibai et al). 1986; **154**:581-90
—— (*see* Tygart et al). 1986; **154**:883-7
Spitz B, Van Assche FA, Vermylen J: Lupus anticcagulant and pregnancy. 1986; **154**:1169 (Letter)
Spivak MM, Librach CL, Rosenthal DM: Microsurgical reversal of sterilization: A six-year study. 1986; **154**: 355-61
Srisuphan W, Bracken MB: Caffeine consumption during pregnancy and association with late spontaneous abortion. 1986; **154**:14-20
—— (*see* Patterson et al). 1986; **154**:1111-2
Stamm WE (*see* Kiviat et al). 1986; **154**:865-73
—— (*see* Paavonen et al). 1986; **154**:556-64
Starr J (*see* Roberts et al). 1986; **154**:206-10
Stedman CM, Baudin JC, White CA, Cooper ES: Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women. 1986; **154**: 1363-9
Steier JA, Sandvei R, Myking OL: Human chorionic gonadotropin in early normal and pathologic pregnancy. 1986; **154**:1091-4
Steigrad SJ (*see* Senior and Steigrad) 1986; **154**:1004-8
Steinberg E (*see* Warford et al). 1986; **154**:21-8
Steingold KA, Judd HL, Nieberg RK, Lu JKH, Chang RJ: Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist. 1986; **154**:1241-8
Stephens JD: Another view of controversy about Rh prophylaxis. 1986; **154**:968-9 (Letter)
Stevens CE (*see* Paavonen et al). 1986; **154**:556-64
Stock RJ: Sterilization failures. 1986; **154**:970 (Letter)
Stranden P (*see* Haukkamaa et al). 1986; **154**:520-4
Strickland DM, Guzick DS, Cox K, Gant NF, Rosenfeld CR: The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy. 1986; **154**:146-8
Stricklin GP, Gast MJ, Welgus HG: Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity. 1986; **154**:134-9
Stubbs TM (*see* Lazarchick et al). 1986; **154**:1050-2
Sueldo CE, Duda M, Kletzky OA: Influence of sequential doses of 5-hydroxytryptophan on prolactin release. 1986; **154**:424-7
Swafford M (*see* Cartwright et al). 1986; **154**:1064-8
Sweet R (*see* Poindexter III et al). 1986; **154**:946-50 (Symposium)
Symonds EM (*see* Powell et al). 1986; **154**:565-9

T

Tabsh K, Rudelstorfer R, Nuwayhid B, Assali NS: Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy. 1986; **154**: 411-9
—— (*see* Rudelstorfer et al). 1986; **154**:462-70
Tamborlane W (*see* Reece et al). 1986; **154**:150-1
Tamura RK (*see* Ory et al). 1986; **154**:1299-1306
Taninecz A (*see* Cartwright et al). 1986; **154**:1064-8
Taslimi MM, Sibai BM, Mason JM, Dacus JV: Immunoglobulin G subclasses and isoimmunized pregnancy outcome. 1986; **154**:1327-32
Taylor PJ, Wiseman D, Mahadevan M, Leader A: "Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions. 1986; **154**:240-4
Taylor SM (*see* Rurak and Taylor). 1986; **154**:674-8
Teasdale F: Relative placental size in growth-retarded infants. 1986; **154**:1170 (Letter reply)
Teisala K (*see* Lehtinen et al). 1986; **154**:158-9
Temple J (*see* Michaels et al). 1986; **154**:537-46
Tennant FR (*see* Zorn et al). 1986; **154**:1234-40
Tessem J (*see* Cotton et al). 1986; **154**:91-3, 1053-9
Testart J (*see* Frydman et al). 1986; **154**:550-5
Thiagarajah S (*see* Bourgeois et al). 1986; **154**:919-20
Thiery M (*see* Defoort and Thiery). 1986; **154**:1169-70 (Letter)
Tho SP (*see* McDonough et al). 1986; **154**:737-48
Thompson K (*see* Dierker Jr et al). 1986; **154**:764-8
Thompson TR (*see* Landrum et al). 1986; **154**:1114-5
Tice T (*see* Erskine et al). 1986; **154**:1109-10
Tischfield JA (*see* McDonough et al). 1986; **154**:737-48
Todd JK (*see* McGregor et al). 1986; **154**:98-103, 109-14
Tomich PB (*see* Arias et al). 1986; **154**:1059-63
Tonetta SA (*see* Ono et al). 1986; **154**:709-16
Torday JS (*see* Barbieri et al). 1986; **154**:1039-43
Torello L (*see* Schmidt et al). 1986; **154**:727-36
Townsend DE: Additional comment on combination conization. 1986; **154**:967 (Letter)
Trill JJ (*see* McDonough et al). 1986; **154**:737-48
Trobough GE (*see* Guderian and Trobough). 1986; **154**:497-503
Tsuchiya A (*see* Roberts et al). 1986; **154**:206-10
Turkel S (*see* Paul et al) 1986; **154**:1187-93
Tustanoff ER (*see* Atack et al). 1986; **154**:287-9
Tuttle D (*see* Cartwright et al). 1986; **154**:1064-8
Tygart SG, McRoyan DK, Spinnato JA, McRoyan CJ, Kitay DZ: Longitudinal study of platelet indices during normal pregnancy. 1986; **154**:883-7

U

Ugalde M (*see* Ono et al). 1986; **154**:709-16
Ujita EL (*see* Ono et al). 1986; **154**:709-16
Unander AM, Lindholm A: Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion. 1986; **154**:516-20
Unis JS (*see* Chambers and Unis). 1986; **154**:1194-9
Unwin W (*see* Apuzzio et al). 1986; **154**:636-7
Usher RH (*see* Bell et al). 1986; **154**:235-9
—— (*see* Bodmer et al). 1986; **154**:244-50

V

Van Assche FA (*see* Spitz et al). 1986; **154**:1169 (Letter)
van de Walle AFGM, Martin CB Jr: Effect of ritodine on uteroplacental blood flow and cardiac output distribution in unanesthetized pregnant guinea pigs. 1986; **154**:189-94
Van Dorsten JP (*see* Lazarchick et al). 1986; **154**:1050-2
Varner MW, Weiner CP, Petzold CR, Galask RP: Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section. 1986; **154**:951-4 (Symposium)
Varner RE (*see* Orr Jr et al). 1986; **154**:960-3 (Symposium)
Vasquez JM (*see* Hull et al). 1986; **154**:1333-8
Veille JC, Bissonnette JM, Hohimer AR: The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat. 1986; **154**:1160-3
Venturoli S (*see* Pasquali et al). 1986; **154**:139-44
Verma RS (*see* Rodriguez et al). 1986; **154**:969-70 (Letter)
Vermylen J (*see* Spitz et al). 1986; **154**:1169 (Letter)
Villanueva AL (*see* Ory et al). 1986; **154**:1299-1306
Villanueva B (*see* Luber et al). 1986; **154**:1264-70
Vintzileos AM (*see* Feinstein et al). 1986; **154**:159-60

Virtanen T (*see* Laatikainen et al). 1986; **154**:94-7
Vorherr H: Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management. 1986; **154**:161-79 (Curr. dev.)
Voto LS (*see* Sánchez et al). 1986; **154**:910-3

W

Wahlström T (*see* Julkunen et al). 1986; **154**:1076-9
Wakim NG (*see* Sanfilippo et al). 1986; **154**:39-43
Walsh SW: Placental prostacyclin production in normal and toxemic pregnancies. 1986; **154**:213-4 (Letter reply)
Wang-Cheng R, Davidson BJ: Ritodrine-induced neutropenia. 1986; **154**:924-5
Warford AL, Levy RA, Rekrut KA, Steinberg E: Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay. 1986; **154**:21-8
Washington SR III (*see* Koerten et al). 1986; **154**:626-8
Wasserheit J (*see* Kiviat et al). 1986; **154**:865-73
Watson P (*see* Prins et al). 1986; **154**:1275-9
Webb T (*see* MacKenzie et al). 1986; **154**:34-9
Weiner CP (*see* Varner et al). 1986; **154**:951-4 (Symposium)
Weiner SA, Lee JKT, Kao M-S, Moon TE: The role of lymphangiography in vulvar carcinoma. 1986; **154**:1073-5
Weiss A (*see* Hart et al). 1986; **154**:878-83
Weiss G, Goldsmith LT, Schoenfeld C, D'Eletto R: Partial purification of relaxin from human seminal plasma. 1986; **154**:749-55
Welgus HG (*see* Stricklin et al). 1986; **154**:134-9
Werlin LB (*see* Schenken et al). 1986; **154**:839-47
Wetrich DW: An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears. 1986; **154**:1339-49
Whalley PJ (*see* Willman et al). 1986; **154**:470-5
White CA (*see* Stedman et al). 1986; **154**:1363-9
White RJ (*see* Frederick et al). 1986; **154**:1116-7
Whitehouse C (*see* MacKenzie et al). 1986; **154**:34-9
Wickham PJD (*see* Bocking et al). 1986; **154**:329-35
Widness JA (*see* Coustan et al) 1986; **154**:1031-5
Wilkins I (*see* Berkowitz et al). 1986; **154**:622-3
Williams ML (*see* Willman et al). 1986; **154**:470-5
Williams RF (*see* Schenken et al). 1986; **154**:839-47
Willman SP, Leveno KJ, Guzick DS, Williams ML, Whalley PJ: Glucose threshold for macrosomia in pregnancy complicated by diabetes. 1986; **154**:470-5
Willson JR: Symposium on the need for flexibility in academic obstetric-gynecologic residencies: Flexibility in obstetric-gynecologic residencies: Why is it necessary? Introduction. 1986; **154**:777-8 (Symposium)
Winkler LS (*see* Greiss Jr et al). 1986; **154**:831-8
Winkler R (*see* Ostrea Jr et al). 1986; **154**:1014-7
Wiseman D (*see* Taylor et al). 1986; **154**:240-4
Wiser WL (*see* Morrison et al). 1986; **154**:900-3
Wiswell R (*see* Artal et al). 1986; **154**:378-83
Wiznitzer A (*see* Mazor et al). 1986; **154**:1119-20
Wølner-Hanssen P (*see* Kiviat et al). 1986; **154**:865-73
Wong PY (*see* Doran et al). 1986; **154**:277-81
Woods DL: Relative placental size in growth-retarded infants. 1986; **154**:1170 (Letter)
Worthington BS (*see* Powell et al). 1986; **154**:565-9

Y

Yancik R, Ries LG, Yates JW: Ovarian cancer in the elderly: An analysis of Surveillance, Epidemiology, and End Results Program data. 1986; **154**:639-47 (Curr. Dev.)
Yandell RB (*see* Moseley et al). 1986; **154**:992-5
Yates JW (*see* Yancik et al). 1986; **154**:639-47 (Curr. Dev.)
Yeast JD (*see* Ray et al). 1986; **154**:1257-63
Yeh M-n (*see* Lee and Yeh). 1986; **154**:1135-9, 1139-46
Yonekura ML (*see* Paul et al) 1986; **154**:1187-93
Young DC (*see* Kogon et al). 1986; **154**:226-30
Young PE (*see* Luber et al). 1986; **154**:1264-70
YoungLai EV (*see* Jarrell et al). 1986; **154**:306-9
Yuen BH (*see* Pride et al). 1986; **154**:1155-60
Yussman MA (*see* Sanfilippo et al). 1986; **154**:39-43

Z

Zabriskie V (*see* Kiviat et al). 1986; **154**:865-73
Zachman RD (*see* Hustead and Zachman). 1986; **154**:203-5
Zadrozny D (*see* Morales et al). 1986; **154**:591-5
Zaltz A (*see* Erskine et al). 1986; **154**:1109-10
Zaroulis CG (*see* Deaver et al). 1986; **154**:153-5
Zinaman M (*see* Barron et al). 1986; **154**:80-4
Zorn EM, Hanson FW, Greve LC, Phelps-Sandall B, Tennant FR: Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis. 1986; **154**:1234-40
Zwolska-Demczuk B (*see* Iffy et al). 1986; **154**:623-4

Subject index*

A

Abdominal hysterectomy
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6

ABO blood group
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9

Abortion
Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis (Zorn et al). 1986; **154**:1234-40
Caffeine consumption during pregnancy and association with late spontaneous abortion (Srisuphan and Bracken). 1986; **154**:14-20 •
Epidemiology of hydatidiform mole during early gestation (Atrash et al). 1986; **154**:906-9
Human chorionic gonadotropin in early normal and pathologic pregnancy (Steier et al). 1986; **154**:1091-4
Occurrence of molar pregnancy in patients undergoing elective abortion: Comparison with other clinical presentations (Ho Yuen and Callegari). 1986; **154**:273-6
The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy (Strickland et al). 1986; **154**:146-8
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20

Acetone
Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios (Hobson et al). 1986; **154**:1023-6

α_1-Acid glycoprotein
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6

Acid-base status
Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings (Page et al). 1986; **154**:1306-11
The relationship between antepartum fetal heart rate, intrapartum fetal heart rate, and fetal acid-base status (Low et al). 1986; **154**:769-76

Acoustic stimulation
Fetal response to vibratory acoustic stimulation in periods of low heart rate reactivity and low activity (Ohel et al). 1986; **154**:619-21

Actin
Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7

Adenocarcinoma; *see also* **Carcinoma**
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22

Adenosine
Maternal ovine placental vascular responses to adenosine (Landauer et al). 1986; **154**:1152-5

Adenosine monophosphate
Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle (Pansini et al). 1986; **154**:679-82

Adhesions
A comparison of three techniques for ovarian reconstruction (Oelsner et al). 1986; **154**:569-72
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4

Adrenal tissue
Prostaglandin secretion by adrenal tissue of human anencephalic fetuses (Carr et al). 1986; **154**:373-8

β-Adrenergic agonist
Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat (Lipshitz et al). 1986; **154**:310-4
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5

*January, 1-220; February, 221-476; March, 477-698; April, 699-972; May, 973-1172; June, 1173-1438.

α-Adrenergic receptor
α-Adrenergic receptors in human myometrium during pregnancy (Berg et al). 1986; **154**:601-6
Human platelet α-adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit (Roberts et al). 1986; **154**:206-10

β-Adrenergic receptor
Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat (Lipshitz et al). 1986; **154**:310-4
Failure to demostrate decreased β-adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition (Dattel et al). 1986; **154**:450-6
Preterm labor: Its diagnosis and management (Gonik and Creasy). 1986; **154**:3-8 (Clin. Opinion)

Adrenocorticotropin
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3

Age
Maternal and fetal surveillance for older pregnant women (Schipul Jr et al) (Letter); (Kirz) (Reply). 1986; **154**: 967-8
Ovarian cancer in the elderly: An analysis of surveillance, epidemiology, and end results program data (Yancik et al). 1986; **154**:639-47 (Curr. Dev.)

Air travel
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000

Alcohol
The effect of maternal consumption of alcohol on human umbilical artery blood flow (Erskine and Ritchie). 1986; **154**:318-21

Alloimmune thrombocytopenic purpura
Neonatal alloimmune thrombocytopenic purpura: A case report (Deaver et al). 1986; **154**:153-5

Ambulatory monitoring
Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery (Katz et al). 1986; **154**:44-7

Amenorrhea
Plasma immunoreactive β-endorphin in exercise-associated amenorrhea (Laatikainen et al). 1986; **154**:94-7

American Gynecological and Obstetrical Society
Transactions of the fourth annual meeting of the American Gynecological and Obstetrical Society. 1986; **154**:699-854

American obstetric and gynecologic societies; *see also* **Papers; Transactions**
Roster of American obstetric and gynecologic societies. 1986; **154**:218-9

Aminophylline
Effect of aminophylline on lung maturation in preterm rabbit fetuses (Cosmi et al). 1986; **154**:436-9

Amniocentesis; *see also* **Amniotic fluid; Chorionic villus**
Amniostat-FLM: An initial clinical trial with both vaginal pool and amniocentesis samples (Benoit et al). 1986; **154**:65-8
Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis (Zorn et al). 1986; **154**:1234-40
Maternal serum α-fetoprotein and fetal autosomal trisomies (Doran et al). 1986; **154**:277-81

Amnion
Epithelial regulation of prolactin effect on amnionic permeability (Raabe and McCoshen). 1986; **154**:130-4

Amniotic fluid
Amniostat-FLM: An initial clinical trial with both vaginal pool and amniocentesis samples (Benoit et al). 1986; **154**:65-8
Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity (Stricklin et al). 1986; **154**:134-9
Amniotic fluid ferning in early gestation (Borten and Friedman). 1986; **154**:628-30
Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity (Kogon et al). 1986; **154**:226-30
Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis (Zorn et al). 1986; **154**:1234-40
Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios (Hobson et al). 1986; **154**:1023-6
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
Further studies on genital mycoplasmas in intra-amniotic infection: Blood cultures and serologic response (Gibbs et al). 1986; **154**:717-26
The in vivo effect of amniotic fluid on endocervical microflora (Patterson et al). 1986; **154**:1111-2
Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy (Pschera et al). 1986; **154**:48-52
Measurements of delta optical density at 450 nm in SS disease (Nelson) (Letter); (Lindsay and Lupo) (Reply). 1986; **154**:1166
A rapid method of culturing bloody amniotic fluid for chromosome analysis (Rodriguez et al) (Letter). 1986; **154**:969-70
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6
Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios (Bastide et al). 1986; **154**:895-900
Use of the drop volume of amniotic fluic in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:63-74

Androgen
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
Testosterone levels and androgen-producing neoplasms (Meldrum) (Letter); (Friedman) (Reply). 1986;**154**:965-6
Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist (Steingold et al). 1986; **154**:1241-8

Anencephaly
Prostaglandin secretion by adrenal tissue of human anencephalic fetuses (Carr et al). 1986; **154**:373-8

Anesthesia
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8

Angiotensin
Fetal responses to maternal infusions of angiotensin II (Perales et al). 1986; **154**:195-203
The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome (Goldkrand and Fuentes). 1986; **154**:792-800
Anomaly; *see also* specific anomaly
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Endometriosis in association with uterine anomaly (Sanfilippo et al). 1986; **154**:39-43
Anterior pituitary; *see* **Pituitary**
Antiandrogenic therapy
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Antibiotics; *see also* specific agent
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8
Antibody
An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody (Hart et al). 1986; **154**:878-83
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1986; **154**:85-90
Mobiluncus-specific antibodies in a postoperative infection (Larsson et al) (Letter). 1986; **154**:1167-8
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20
Anticoagulant
Lupus anticoagulant and pregnancy (Spitz et al) (Letter). 1986; **154**:1169
Antigen
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9
Neonatal alloimmune thrombocytopenic purpura: A case report (Deaver et al). 1986; **154**:153-5
Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department (Malecki et al). 1986; **154**:625-6
Anti-Kell antibody
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1986; **154**:85-90
Antiplatelet antibody
An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody (Hart et al). 1986; **154**:878-83
Anxiety
Postpartum moods in men and women (Quadagno et al). 1986; **154**:1018-23
Aorta
Aortic function during normal human pregnancy (Hart et al). 1986; **154**:887-91
Apgar score
Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings (Page et al). 1986; **154**:1306-11
Appendix
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
Arginine
Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep (Newnham et al). 1986; **154**:663-6
Sodium excretion in human pregnancy: A role for arginine vasopressin (Brown and Gallery). 1986; **154**:914-9
Asphyxia
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**:235-9
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Autoimmune disease
Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura (Meschengieser and Lazzari) (Letter); (Martin Jr) (Reply). 1986; **154**:1166-7
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20
Autosomal trisomy
Maternal serum α-fetoprotein and fetal autosomal trisomies (Doran et al). 1986; **154**:277-81

B

Bacteria; *see also* **Flora; Infection**
Bacterial flora of the cervix in women using different methods of contraception (Haukkamaa et al). 1986; **154**:520-4
Etiology of cervical inflammation (Paavonen et al). 1986; **154**:556-64
Barbiturates
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8
Behavior
The effect of electrical stimulation on behavioral states in the fetal lamb (Natale and Nasello-Paterson). 1986; **154**:321-8
Biofeedback
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Biophysical profile scoring
Biophysical profile scoring in the management of the postterm pregnancy: An analysis of 307 patients (Johnson et al). 1986; **154**:269-73
Biopsy
Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear (Burk et al). 1986; **154**:982-9
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5
Birth; *see* **Delivery**
Birth injury
Fetal injury prior to labor: Does it happen? (Paul et al) 1986; **154**:1187-93
Perinatal events and cerebral palsy (Hensleigh et al). 1986; **154**:978-81

Birth weight
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Prepregnancy weight, weight gain, and birth weight (Abrams and Laros Jr). 1986; **154**:503-9
Bladder sphincter
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Bleeding; *see* **Hemorrhage**
Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8
Blood group
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9
Blood sampling
Percutaneous umbilical sampling in immune thrombocytopenic purpura (Sacks) (Letter); (Hobbins et al) (Reply). 1986; **154**:214-5
Blood transfusion
Blood transfusions and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology (Sacks and Koppes). 1986; **154**:483-6 (Clin. Opinion)
Blood volume expansion
Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion (Cotton et al). 1986; **154**:1053-9
Books received
Books received. 1986; **154**:696, 971, 1171, 1370
Bowel obstruction
Disagreement on use of water-soluble contrast material in small bowel obstruction (Ott et al) (Letter); (Robertson) (Reply). 1986; **154**:690-1
Postoperative intussusception after vaginal vault suspension (Hunter et al). 1986; **154**:633-4
Breast
Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management (Vorherr). 1986; **154**:161-79
Lactation suppression and puerperal fever (Almeida and Kitay). 1986; **154**:940-1
On the origin and progression of human breast cancer (Jensen). 1986; **154**:1280-4
Breast-feeding
Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura (Meschengieser and Lazzari) (Letter); (Martin Jr) (Reply). 1986; **154**:1166-7
Excretion of varicella–herpes zoster virus in breast milk (Frederick et al). 1986; **154**:1116-7
Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant (Ostrea Jr et al). 1986; **154**:1014-7
Breech presentation
Abdominal rescue after entrapment of the aftercoming head (Iffy et al). 1986; **154**:623-4
External cephalic version of the breech presentation under tocolysis (Morrison et al). 1986; **154**:900-3
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
The length of the human umbilical cord in vertex and breech presentations (Soernes and Bakke). 1986; **154**:1086-7
Bromocriptine
Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast (Ho Yuen et al). 1986; **154**:336-40
Lactation suppression and puerperal fever (Almeida and Kitay). 1986; **154**:940-1
Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia (Kletzky et al). 1986; **154**:431-5

C

CA 125
CA 125 surveillance and second-look laparotomy in ovarian carcinoma (Atack et al). 1986; **154**:287-9
The tumor marker CA 125 is a common constituent of normal cervical mucus (de Bruijn et al). 1986; **154**:1088-91
Caffeine
Caffeine consumption during pregnancy and association with late spontaneous abortion (Srisuphan and Bracken). 1986; **154**:14-20
Calcium channel blocker
The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat (Veille et al). 1986; **154**:1160-3
Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7
Cannulas
A randomized study of three cannulas for transcervical chorionic villus sampling (MacKenzie et al). 1986; **154**:34-9
Carbon dioxide laser
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Carcinoma; *see also* **Neoplasia**
CA 125 surveillance and second-look laparotomy in ovarian carcinoma (Atack et al). 1986; **154**:287-9
Cervical carcinoma in women aged 34 and younger (Carmichael et al). 1986; **154**:264-9
Cigarette smoking and endometrial cancer (Baron) (Letter). 1986; **154**:965
The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells (Moise et al). 1986; **154**:1080-5
Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites (Lancaster et al). 1986; **154**:115-9
On the origin and progression of human breast cancer (Jensen). 1986; **154**:1280-4
Ovarian cancer in the elderly: An analysis of surveillance, epidemiology, and end results program data (Yancik et al). 1986; **154**:639-47 (Curr. Dev.)
Ovarian carcinoma of low malignant potential: Staging and treatment (Nation and Krepart). 1986; **154**:290-3
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
Primary carcinoma of the fallopian tube (Podratz et al). 1986; **154**:1319-26
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22
The role of lymphangiography in vulvar carcinoma (Weiner et al). 1986; **154**:1073-5
Staging laparotomy in early epithelial ovarian carcinoma (Helewa et al). 1986; **154**:282-6

Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6

Cardiovascular disease; *see* Heart

β-Carotene
Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant (Ostrea Jr et al). 1986; **154**:1014-7

Case reports
The value of case reports in human teratology (Goldberg and Golbus). 1986; **154**:479-82 (Clin. Opinion)

Catecholamine
Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy (Pschera et al). 1986; **154**:48-52
Plasma catecholamine responses to physiologic stimuli in normal human pregnancy (Barron et al). 1986; **154**: 80-4

Cefotetan
Cefotetan in the treatment of obstetric and gynecologic infections (Poindexter III et al). 1986; **154**:946-50 (Symposium)
Cefotetan versus cefoxitin as prophylaxis in hysterectomy (Orr Jr et al). 1986; **154**:960-3 (Symposium)
Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section (Varner et al). 1986; **154**:951-4 (Symposium)
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)
Symposium on Cefotetan: Introduction (Quintiliani). 1986; **154**:945 (Symposium)

Cefoxitin
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8
Cefotetan versus cefoxitin as prophylaxis in hysterectomy (Orr Jr et al). 1986; **154**:960-3 (Symposium)
Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section (Varner et al). 1986; **154**:951-4 (Symposium)
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)

Central Association of Obstetricians and Gynecologists
Transactions of the fifty-third annual meeting of the Central Association of Obstetricians and Gynecologists. 1986; **154**:1285-1369

Cephalic version
External cephalic version of the breech presentation under tocolysis (Morrison et al). 1986; **154**:900-3

Cephalopelvic disproportion
Factors related to the increasing cesarean section rates for cephalopelvic disproportion (Silbar). 1986; **154**: 1095-8

Cephradine
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8

Cerebral palsy
Perinatal events and cerebral palsy (Hensleigh et al). 1986; **154**:978-81

Cervical factor
Experience with intrauterine insemination for cervical factor and oligospermia (Hull et al). 1986; **154**:1333-8

Cervix
An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears (Wetrich). 1986; **154**:1339-49
Bacterial flora of the cervix in women using different methods of contraception (Haukkamaa et al). 1986; **154**: 520-4
Cervical carcinoma in women aged 34 and younger (Carmichael et al). 1986; **154**:264-9
Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods (Falcone and Ferenczy). 1986; **154**:260-4
Etiology of cervical inflammation (Paavonen et al). 1986; **154**:556-64
Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites (Lancaster et al). 1986; **154**:115-9
Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear (Burk et al). 1986; **154**:982-9
The in vivo effect of amniotic fluid on endocervical microflora (Patterson et al). 1986; **154**:1111-2
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5
Preinduction cervical ripening with sequential use of prostaglandin E_2 gel (Prins et al). 1986; **154**:1275-9
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6
Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases (Deigen et al). 1986; **154**:255-9
The tumor marker CA 125 is a common constituent of normal cervical mucus (de Bruijn et al). 1986; **154**:1088-91
Ultrasound differentiation of the competent from the incompetent cervix: Prevention of preterm delivery (Michaels et al). 1986; **154**:537-46

Cesarean section
Abdominal rescue after entrapment of the aftercoming head (Iffy et al). 1986; **154**:623-4
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**: 235-9
Cesarean delivery of the second twin after vaginal delivery of the first twin (Rattan et al). 1986; **154**:936-9
Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section (Varner et al). 1986; **154**:951-4 (Symposium)
Factors related to the increasing cesarean section rates for cephalopelvic disproportion (Silbar). 1986; **154**: 1095-8
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)
Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section (Ganesh et al). 1986; **154**:1113-4

Change
Change and consistency (Brewer et al). 1986; **154**:1-2 (Editorial)

Chemotherapy
Ovarian carcinoma of low malignant potential: Staging and treatment (Nation and Krepart). 1986; **154**:290-3
Chlamydia trachomatis
Etiology of cervical inflammation (Paavonen et al). 1986; **154**:556-64
Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease (Kiviat et al). 1986; **154**:865-73
Residues of pelvic inflammatory disease in intrauterine device users: A result of the intrauterine device or *Chlamydia trachomatis* infection? (Guderian and Trobough). 1986; **154**:497-503
Choriocarcinoma
Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line (Sarkar et al). 1986; **154**:390-3
The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells (Moise et al). 1986; **154**:1080-5
Chorionic gonadotropin; *see* **Gonadotropin**
Chorionic villus; *see also* **Amniocentesis**
Chorionic villus sampling: Experience of the first 1000 cases (Hogge et al). 1986; **154**:1249-52
Chorionic villus sampling in continuing pregnancies. I. Low fetal loss rates in initial 109 cases (Elias et al). 1986; **154**:1349-52
Chorionic villus sampling in continuing pregnancies. II. Cytogenetic reliability (Martin et al). 1986; **154**:1353-62
Improving the yield of direct chorionic villus slide preparations (Bhatia et al). 1986; **154**:408-11
A randomized study of three cannulas for transcervical chorionic villus sampling (MacKenzie et al). 1986; **154**:34-9
Septic shock with renal failure after chorionic villus sampling (Barela et al). 1986; **154**:1100-2
Chromosome
Hydrops fetalis and chromosomal trisomies (Landrum et al). 1986; **154**:1114-5
A rapid method of culturing bloody amniotic fluid for chromosome analysis (Rodriguez et al) (Letter). 1986; **154**:969-70
Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes (McDonough et al). 1986; **154**:737-48
Cigarette smoking
Cigarette smoking and endometrial cancer (Baron) (Letter). 1986; **154**:965
Circulation
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
Clear cell adenocarcinoma
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22
Clinical Opinion
Clinical Opinion. 1986; **154**:3-13, 479-86, 973-81
Clomiphene citrate
The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo (Schmidt et al). 1986; **154**:727-36
Colectomy
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
Collagenase inhibitor
Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity (Stricklin et al). 1986; **154**:134-9
Colonization; *see* **Flora**
Microbial colonization of tailed and tailless intrauterine contraceptive devices: Influence of the mode of insertion in the rabbit (Jacques et al). 1986; **154**:648-55
Colpocleisis
Partial colpocleisis (Langmade and Oliver Jr). 1986; **154**:1200-5
Colposcopy
An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears (Wetrich). 1986; **154**:1339-49
Behavior of the squamocolumnar junction during normal pregnancy (Ganesh et al) (Letter). 1986; **154**:689-90
Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods (Falcone and Ferenczy). 1986; **154**:260-4
Colposcopy in the diagnosis of penile condyloma (Sedlacek et al). 1986; **154**:494-6
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5
Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases (Deigen et al). 1986; **154**:255-9
Condyloma
Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods (Falcone and Ferenczy). 1986; **154**:260-4
Colposcopy in the diagnosis of penile condyloma (Sedlacek et al). 1986; **154**:494-6
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Congenital anomaly; *see also* **specific anomaly**
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Conization
Additional comment on combination conization (Townsend). 1986; **154**:967
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5
Conjugated equine estrogen
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:456-61
Consistency
Change and consistency (Brewer et al). 1986; **154**:1-2 (Editorial)
Constrictive pericarditis
Constrictive pericarditis and pregnancy (Sachs et al). 1986; **154**:156-7
Contraception; *see also* **Intrauterine device**
Bacterial flora of the cervix in women using different methods of contraception (Haukkamaa et al). 1986; **154**:520-4

Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Toxic shock syndrome, contraceptive methods, and vaginitis (Lanes et al). 1986; **154**:989-91

Contrast material
Disagreement on use of water-soluble contrast material in small bowel obstruction (Ott et al) (Letter); (Robertson) (Reply). 1986; **154**:690-1

Coronary artery disease
Coronary artery disease in diabetic pregnancies (Reece et al). 1986; **154**:150-1
Estrogen use and cardiovascular disease (Henderson et al). 1986; **154**:1181-6

Corpus luteum
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63
Ovarian pregnancy with a contralateral corpus luteum: Case report (Check and Chase). 1986; **154**:155-6

Correspondence
Letters. 1986; **154**:211-7, 689-95, 964-70, 1164-70

Corticotropin
Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion (Blumenfeld et al). 1986; **154**:606-12

Cortisol
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3

C-peptide
Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy (Pschera et al). 1986, **154**:48-52

C-reactive protein
Serum C-reactive protein determination in acute pelvic inflammatory disease (Lehtinen et al). 1986; **154**:158-9

Cultures
An evaluation of different media for the zona-free hamster egg penetration test (Mortimer). 1986; **154**:351-4
Further studies on genital mycoplasms in intra-amniotic infection: Blood cultures and serologic response (Gibbs et al). 1986; **154**:717-26
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
A rapid method of culturing bloody amniotic fluid for chromosome analysis (Rodriguez et al) (Letter). 1986 **154**:969-70

Curettage
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5

Current development
Current development. 1986; **154**:639-47, 1121-32

Cushing's syndrome
Cushing's syndrome in pregnancy: A case report and literature review (Koerten et al). 1986; **154**:626-8

Cyclic adenosine monophosphate
Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle (Pansini et al). 1986; **154**:579-82

Cyproterone acetate
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44

Cystic hygroma colli
Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management (Garden et al). 1986; **154**:221-5

Cystourethropexy
Modified retropubic cystourethropexy (Goodno Jr and Powers). 1986; **154**:1211-5

D

Danazol
Inhibition of gonadotropin-releasing hormone receptors in rat anterior pituitary monolayer cell cultures by danazol (Menon et al). 1986; **154**:362-72
Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit (Pride et al). 1986; **154**:1155-60

Death; *see* Mortality

Dehydroepiandrosterone sulfate
The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells (Moise et al). 1986; **154**:1080-5

Delivery
Abdominal rescue after entrapment of the aftercoming head (Iffy et al). 1986; **154**:623-4
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**:235-9
Cesarean delivery of the second twin after vaginal delivery of the first twin (Rattan et al). 1986; **154**:936-9
Failure to demostrate decreased β-adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition (Dattel et al). 1986; **154**:450-6
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Midforceps deliveries: Long-term outcome of infants (Dierker Jr et al). 1986; **154**:764-8
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3
Reply to Dr. Emanuel Friedman (Kadar) (Letter). 1986; **154**:694-5
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9

Deoxyribonucleic acid
Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites (Lancaster et al). 1986; **154**:115-9
Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes (McDonough et al). 1986; **154**:737-48

Desensitization
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5

Development
The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study (Shime et al). 1986; **154**:341-5

Dexamethasone
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5
The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with premature rupture of membranes (Morales et al). 1986; **154**:591-5
Diabetes
Coronary artery disease in diabetic pregnancies (Reece et al). 1986; **154**:150-1
The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman (Ray et al). 1986; **154**:1257-63
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
Fetal α-melanocyte–stimulating homone levels: No correlation with late fetal growth but increased with diabetes mellitus (Hassan et al). 1986; **154**:428-30
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Glucose threshold for macrosomia in pregnancy complicated by diabetes (Willman et al). 1986; **154**:470-5
Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy (Pschera et al). 1986; **154**:48-52
Serum fructosamine: A screening test for diabetes in pregnancy (Roberts and Baker). 1986; **154**:1027-30
Should a fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? (Coustan et al) 1986; **154**:1031-5
Diagnosis related groups
Obstetric hospital stays by diagnosis related groups: A community-based analysis (Lagoe). 1986; **154**:873-8
Diet
Effect of long-term administration of β_2-sympathomimetic drug in the diet-restricted pregnant rat model (Lipshitz et al). 1986; **154**:314-7
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Diethylstilbestrol
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22
Treatment of endometriosis (Lockhart Jr) (Letter). 1986; **154**:215-6
Upper genital tract changes and infertility in diethylstilbestrol-exposed women (Kaufman et al). 1986; **154**:1312-8
Dopamine
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
Modulation of luteinizing hormone immunoreactivity and bioactivity by dopamine but not norepinephrine in women (Barnes et al). 1986; **154**:445-50
Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia (Kletzky et al). 1986; **154**:431-5
Variability and selectivity of anterior pituitary response to dopamine agonists throughout the normal menstrual cycle (Kletzky and Shangold). 1986; **154**:362-7
Doppler measurement
Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death (Erskine et al). 1986; **154**:1109-10
Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth (Shime et al). 1986; **154**:294-300
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13
Down's syndrome
A sonographic sign for fetal Down's syndrome (Defoort and Thiery) (Letter). 1986; **154**:1169-70
Drop volume
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74
Dysmaturity
The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study (Shime et al). 1986; **154**:341-5
Dyspareunia
Focal vulvitis: A characteristic syndrome and cause of dyspareunia: Features, natural history, and management (Peckham et al). 1986; **154**:855-64
Dystocia
Another technique for resolution of shoulder dystocia (Carter) (Letter). 1986; **154**:964

E

Echocardiography
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth (Shime et al). 1986; **154**:294-300
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3
Eclampsia; *see also* **Preeclampsia**
Eclampsia. V. The incidence of nonpreventable eclampsia (Sibai et al). 1986; **154**:581-90
Hypertensive diseases of pregnancy and parity (Gleicher et al). 1986; **154**:1044-9
Ectopic pregnancy
Conservative treatment of ectopic pregnancy with methotrexate (Ory et al). 1986; **154**:1299-1306
Tubal conservation in ectopic pregnancy: A study of 200 cases (Hallatt). 1986; **154**:1216-21
Edema
Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Goodlin). 1986; **154**:634-5
Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema (Cotton et al). 1986; **154**:91-3
Editorials
Editorial. 1986; **154**:1-2, 477-8
Education; *see also* **Residency**
The Golden Thread (Malkasian Jr). 1986; **154**:1285-90 (Pres. address)
The need for flexibility in preparing clinician-scientists for academic careers (Jaffe). 1986; **154**:778-80 (Symposium)

Egg penetration test
An evaluation of different media for the zona-free hamster egg penetration test (Mortimer). 1986; **154**:551-4
Elderly; *see* **Age**
Electrical stimulation
The effect of electrical stimulation on behavioral states in the fetal lamb (Natale and Nasello-Paterson). 1986; **154**:321-8
Electrocardiogram
Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia (Hendricks et al). 1986; **154**:921-3
Electrocautery
Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases (Deigen et al). 1986; **154**:255-9
Embryo; *see also* **Embryo transfer**
The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo (Schmidt et al). 1986; **154**:727-36
Embryo transfer; *see also* **Fertilization**
An obstetric assessment of the first 100 births from the in vitro fertilization program at Clamart, France (Frydman et al). 1986; **154**:550-5
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4
An in vitro fertilization and embryo transfer pilot study: Treatment-dependent and treatment-independent pregnancies (Jarrell et al). 1986; **154**:231-4
Emphysema
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Encephalitis
Meningoencephalitis caused by *Histoplasma capsulatum* complicating pregnancy (McGregor et al). 1986; **154**:925-31
Enclomiphene citrate
The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo (Schmidt et al). 1986; **154**:727-36
Endocarditis
Infective endocarditis in obstetric and gynecologic practice (Seaworth and Durack). 1986; **154**:180-8
Endocervix; *see also* **Cervix**
The in vivo effect of amniotic fluid on endocervical microflora (Patterson et al). 1986; **154**:1111-2
Necessity of endocervical curettage in colposcopy (Moseley et al). 1986; **154**:992-5
Endometriosis
Analysis of sequential treatment protocols for endometriosis-associated infertility (Olive and Lee). 1986; **154**:613-9
Endometriosis in association with uterine anomaly (Sanfilippo et al). 1986; **154**:39-43
Family trait analysis: A case-control study of 43 women with endometriosis and their best friends (Lamb et al). 1986; **154**:596-601
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay (Chacho et al). 1986; **154**:1290-9
Prostaglandins and endometriosis (Sgarlata and Hertelendy) (Letter); (James) (Reply). 1986; **154**:964-5
Treatment of endometriosis (Lockhart Jr) (Letter). 1986; **154**:215-6
Endometritis
Cefotetan in the treatment of obstetric and gynecologic infections (Poindexter III et al). 1986; **154**:946-50 (Symposium)
Serum C-reactive protein determination in acute pelvic inflammatory disease (Lehtinen et al). 1986; **154**:158-9
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)
Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section (Ganesh et al). 1986; **154**:1113-4
Endometrium; *see also* **Endometriosis; Endometritis**
Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle (Pansini et al). 1986; **154**:679-82
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:456-61
Cigarette smoking and endometrial cancer (Baron) (Letter). 1986; **154**:965
β-Endorphin
Plasma immunoreactive β-endorphin in exercise-associated amenorrhea (Laatikainen et al). 1986; **154**:94-7
Engorgement
Lactation suppression and puerperal fever (Almeida and Kitay). 1986; **154**:940-1
Entrapment of aftercoming head
Abdominal rescue after entrapment of the aftercoming head (Iffy et al). 1986; **154**:623-4
Enzyme-linked immunosorbent assay
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Epinephrine
Plasma catecholamine responses to physiologic stimuli in normal human pregnancy (Barron et al). 1986; **154**:80-4
Epithelium
Epithelial regulation of prolactin effect on amnionic permeability (Raabe and McCoshen). 1986; **154**:130-4
Errata
Alloimmunization in twin pregnancies (Bowman) (1985; **153**:10-1). 1986; **154**:217
Heat flux and oxygen consumption of the pregnant uterus (1986; **154**:462-9). 1986; **154**:695
Erythroblastosis fetalis; *see* **Rh isoimmunization**
Erythrocyte
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9
Erythromycin
Adjunctive erythomycin treatment for idiopathic preterm labor: Results of a randomized, double-blinded, placebo-controlled trial (McGregor et al). 1986; **154**:98-103
Escherichia coli
Vaginal colonization with *Escherichia coli* in healthy women (Chow et al). 1986; **154**:120-6
Estriol
The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman (Ray et al). 1986; **154**:1257-63

Estrogen
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:456-61
Estrogen use and cardiovascular disease (Henderson et al). 1986; **154**:1181-6
Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management (Vorherr). 1986; **154**:161-79
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63
Temporal and receptor correlates of the estrogen response in sheep (Greiss Jr et al). 1986; **154**:831-8
Treatment of endometriosis (Lockhart Jr) (Letter). 1986; **154**:215-6

Ethics
The Golden Thread (Malkasian Jr). 1986; **154**:1285-90 (Pres. address)
Your morality or mine? An inquiry into the ethics of human reproduction (Hill). 1986; **154**:1173-80 (Pres. address)

Ethinyl estradiol
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44

Exchange transfusion
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74

Exercise
Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods (Notelovitz et al). 1986; **154**:1009-13
Moderate recreational running is associated with lowered salivary progesterone profiles in women (Ellison and Lager). 1986; **154**:1000-3
Plasma immunoreactive β-endorphin in exercise-associated amenorrhea (Laatikainen et al). 1986; **154**:94-7
Pulmonary responses to exercise in pregnancy (Artal et al). 1986; **154**:378-83
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64

External cephalic version
External cephalic version of the breech presentation under tocolysis (Morrison et al). 1986; **154**:900-3

Extracorporeal perfusion
Extracorporeal perfusion of the human uterus (Bulletti et al). 1986; **154**:683-8

F

Fallopian tube
Human fallopian tube contains placental protein 14 (Julkunen et al). 1986; **154**:1076-9
Primary carcinoma of the fallopian tube (Podratz et al). 1986; **154**:1319-26
Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization (Luber et al). 1986; **154**:1264-70
Tubal conservation in ectopic pregnancy: A study of 200 cases (Hallatt). 1986; **154**:1216-21

Family trait analysis
Family trait analysis: A case-control study of 43 women with endometriosis and their best friends (Lamb et al). 1986; **154**:596-601

Ferning
Amniotic fluid ferning in early gestation (Borten and Friedman). 1986; **154**:628-30

Fertility; *see* **Infertility**

Fertilization; *see also* **Embryo transfer; Infertility**
An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries (Andrews et al). 1986; **154**:848-54
The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo (Schmidt et al). 1986; **154**:727-36
Experience with intrauterine insemination for cervical factor and oligospermia (Hull et al). 1986; **154**:1333-8
An in vitro fertilization and embryo transfer pilot study: Treatment-dependent and treatment-independent pregnancies (Jarrell et al). 1986; **154**:231-4
An obstetric assessment of the first 100 births from the in vitro fertilization program at Clamart, France (Frydman et al). 1986; **154**:550-5
Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization (Luber et al). 1986; **154**:1264-70
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4

Fetal heart rate
Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings (Page et al). 1986; **154**:1306-11
Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep (Bocking et al). 1986; **154**:329-35
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**:250-4
Fetal injury prior to labor: Does it happen? (Paul et al) 1986; **154**:1187-93
Fetal response to vibratory acoustic stimulation in periods of low heart rate reactivity and low activity (Ohel et al). 1986; **154**:619-21
The relationship between antepartum fetal heart rate, intrapartum fetal heart rate, and fetal acid-base status (Low et al). 1986; **154**:769-76
Relationship of baseline fetal heart rate to gestational age and fetal sex (Druzin et al). 1986; **154**:1102-3
Sequential nonstress testing with use of each fetus as its own control (Devoe et al). 1986; **154**:931-6
Sinusoidal fetal heart rate pattern after administration of nalbuphine hydrochloride: A case report (Feinstein et al). 1986; **154**:159-60

Fetal lung maturity
Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity (Stricklin et al). 1986; **154**:134-9
Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity (Kogon et al). 1986; **154**:226-30
Effect of aminophylline on lung maturation in preterm rabbit fetuses (Cosmi et al). 1986; **154**:436-9
Effect of induced oligohydramnios on fetal lung development (King et al). 1986; **154**:823-30
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74

Fetomaternal hemorrhage
Fetomaternal hemorrhage associated with umbilical vein thrombosis (Hoag). 1986; **154**:1271-4
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9
α-Fetoprotein
Maternal serum α-fetoprotein and fetal autosomal trisomies (Doran et al). 1986; **154**:277-81
Fetus; *see also* **Chorionic villus; Fetal heart rate; Fetal lung maturity; Fetomaternal hemorrhage**
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion (Blumenfeld et al). 1986; **154**:606-12
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5
The effect of electrical stimulation on behavioral states in the fetal lamb (Natale and Nasello-Paterson). 1986; **154**:321-8
Effect of maternal hyperketonemia in hyperglycemic pregnant ewes and their fetuses (Miodovnik et al). 1986; **154**:394-401
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74
Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep (Bocking and Harding). 1986; **154**:655-62
Extracorporeal perfusion of the human uterus (Bulletti et al). 1986; **154**:683-8
Factors influencing nonstress test results in multiple gestations (Patkos et al). 1986; **154**:1107-8
Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death (Erskine et al). 1986; **154**:1109-10
Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management (Garden et al). 1986; **154**:221-5
Fetal injury prior to labor: Does it happen? (Paul et al) 1986; **154**:1187-93
Fetal α-melanocyte–stimulating homone levels: No correlation with late fetal growth but increased with diabetes mellitus (Hassan et al). 1986; **154**:428-30
Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus (Lee and Yeh). 1986; **154**:1133-9
Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis (Lee and Yeh). 1986; **154**:1139-46
Fetal responses to maternal infusions of angiotensin II (Perales et al). 1986; **154**:195-203
Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9
Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy (Dorko and Hayashi). 1986; **154**:801-5
Intravascular transfusion in utero: The percutaneous approach (Berkowitz et al). 1986; **154**:622-3
The length of the human umbilical cord in vertex and breech presentations (Soernes and Bakke). 1986; **154**:1086-7
Maternal, fetal, and newborn complications associated with newborn intracranial hemorrhage (Low et al). 1986; **154**:345-51
Maternal and fetal surveillance for older pregnant women (Schipul Jr et al) (Letter); (Kirz) (Reply). 1986; **154**:967-8
Maternal ovine placental vascular responses to adenosine (Landauer et al). 1986; **154**:1152-5
Maternal serum α-fetoprotein and fetal autosomal trisomies (Doran et al). 1986; **154**:277-81
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3
The prenatal diagnosis of Robin anomalad (Pilu et al). 1986; **154**:630-2
Prostaglandin secretion by adrenal tissue of human anencephalic fetuses (Carr et al). 1986; **154**:373-8
Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth (Shime et al). 1986; **154**:294-300
Relative placental size in growth-retarded infants (Woods) (Letter); (Teasdale) (Reply). 1986; **154**:1170
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22
A sonographic sign for fetal Down's syndrome (Defoort and Thiery) (Letter). 1986; **154**:1169-70
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
Fever
Lactation suppression and puerperal fever (Almeida and Kitay). 1986; **154**:940-1
Fibrocystic breast disease
Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management (Vorherr). 1986; **154**:161-79
Fibronectin
Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic (Lazarchick et al). 1986; **154**:1050-2
Fistula
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6
Flora
Bacterial flora of the cervix in women using different methods of contraception (Haukkamaa et al). 1986; **154**:520-4
Microbial colonization of tailed and tailless intrauterine contraceptive devices: Influence of the mode of insertion in the rabbit (Jacques et al). 1986; **154**:648-55

Vaginal colonization with *Escherichia coli* in healthy women (Chow et al). 1986; **154**:120-6
The in vivo effect of amniotic fluid on endocervical microflora (Patterson et al). 1986; **154**:1111-2

Fluosol-DA
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74

Focal vulvitis
Focal vulvitis: A characteristic syndrome and cause of dyspareunia: Features, natural history, and management (Peckham et al). 1986; **154**:855-64

Folic acid
Folate deficiency presenting as pancytopenia in pregnancy (Solano Jr and Councell). 1986; **154**:1117-8

Follicle
An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels (Jarrell et al). 1986; **154**:306-9
Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis (Ono et al). 1986; **154**:709-16
Histologic and hormonal documentation of the luteinized unruptured follicle syndrome (Schenken et al). 1986; **154**:839-47

Forceps
Reply to Dr. Emanuel Friedman (Kadar) (Letter). 1986; **154**:694-5

Fructosamine
Serum fructosamine: A screening test for diabetes in pregnancy (Roberts and Baker). 1986; **154**:1027-30

G

Gaucher's disease
Gaucher's disease in pregnancy associated with portal hypertension (Mazor et al). 1986; **154**:1119-20

Gender
Relationship of baseline fetal heart rate to gestational age and fetal sex (Druzin et al). 1986; **154**:1102-3

Genetics
Family trait analysis: A case-control study of 43 women with endometriosis and their best friends (Lamb et al). 1986; **154**:596-601

Genital herpes
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8

Gestational age; *see also* Prematurity
Amniotic fluid collagenase inhibitor: Correlation with gestational age and fetal lung maturity (Stricklin et al). 1986; **154**:134-9

Gestational diabetes; *see* Diabetes

Gestosis
Expanded toxemia syndrome or gestosis (Goodlin). 1986; **154**:1227-33

Glucocorticosteroid
Prostaglandin secretion by adrenal tissue of human anencephalic fetuses (Carr et al). 1986; **154**:373-8

Glucose; *see also* Diabetes
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Glucose threshold for macrosomia in pregnancy complicated by diabetes (Willman et al). 1986; **154**:470-5
Should a fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? (Coustan et al) 1986; **154**:1031-5
Uterine metabolism of the pregnant rabbit under chronic steady-state conditions (Johnson et al). 1986; **154**:1146-51

Glycoprotein
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6

Goiter
Goiter in pregnancy (Hegedüs et al) (Letter); (Long et al) (Reply). 1986; **154**:1164

Gonadotropin
An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels (Jarrell et al). 1986; **154**:306-9
Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion (Blumenfeld et al). 1986; **154**:606-12
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
Human chorionic gonadotropin in early normal and pathologic pregnancy (Steier et al). 1986; **154**:1091-4
Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast (Ho Yuen et al). 1986; **154**:336-40
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Relationship between log of the human chorionic gonadotropin concentration and time period in early pregnancy (Kadar) (Letter); (Pittaway) (Reply). 1986; **154**:692-4

Gonadotropin-releasing hormone
Inhibition of gonadotropin-releasing hormone receptors in rat anterior pituitary monolayer cell cultures by danazol (Menon et al). 1986; **154**:362-72
Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit (Pride et al). 1986; **154**:1155-60
Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist (Steingold et al). 1986; **154**:1241-8

Graft
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6

Granulosa cell
Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis (Ono et al). 1986; **154**:709-16

Group B β-hemolytic streptococcal sepsis
An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis (Minkoff and Mead). 1986; **154**:973-7

Growth hormone
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3

Growth retardation
Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus (Lee and Yeh). 1986; **154**:1133-9
Relative placental size in growth-retarded infants (Woods) (Letter); (Teasdale) (Reply). 1986; **154**:1170

H

Hamman's syndrome
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Heart
Aortic function during normal human pregnancy (Hart et al). 1986; **154**:887-91
Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods (Notelovitz et al). 1986; **154**:1009-13
Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion (Cotton et al). 1986; **154**:1053-9
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Coronary artery disease in diabetic pregnancies (Reece et al). 1986; **154**:150-1
Effect of ritodrine on uteroplacental blood flow and cardiac output distribution in unanesthetized pregnant guinea pigs (van de Walle and Martin Jr). 1986; **154**:189-94
Estrogen use and cardiovascular disease (Henderson et al). 1986; **154**:1181-6
Multiple peripheral pulmonic stenosis in pregnancy (Landsberger and Grossman III). 1986; **154**:152-3
Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth (Shime et al). 1986; **154**:294-300
Sinusoidal fetal heart rate pattern after administration of nalbuphine hydrochloride: A case report (Feinstein et al). 1986; **154**:159-60
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3
Heat flux
Heat flux and oxygen consumption of the pregnant uterus (Rudelstorfer et al). 1986; **154**:462-70
Hegar's sign
Correct use of Hegar's sign (Speck) (Letter); (Munsick) (Reply). 1986; **154**:691-2
HELLP syndrome
Severe hypoglycemia associated with the HELLP syndrome (Mabie and Sibai) (Letter); (Egley) (Reply). 1986; **154**:211-2
Hematocolpos
Endometriosis in association with uterine anomaly (Sanfilippo et al). 1986; **154**:39-43
Hematocrit
Human platelet α-adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit (Roberts et al). 1986; **154**:206-10
Hemicolectomy
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
Hemolytic disease of neonate; *see* **Rh isoimmunization**
β-Hemolytic streptococcal sepsis
An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis (Minkoff and Mead). 1986; **154**:973-7
Hemorrhage
Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage (Shankaran et al). 1986; **154**:53-7
Fetomaternal hemorrhage associated with umbilical vein thrombosis (Hoag). 1986; **154**:1271-4
Is there a causal connection between motile rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8
Maternal, fetal, and newborn complications associated with newborn intracranial hemorrhage (Low et al). 1986; **154**:345-51
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9
Hepatitis
Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department (Malecki et al). 1986; **154**:625-6
Heredity
Family trait analysis: A case-control study of 43 women with endometriosis and their best friends (Lamb et al). 1986; **154**:596-601
Herpes simplex virus
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Herpes zoster virus
Excretion of varicella–herpes zoster virus in breast milk (Frederick et al). 1986; **154**:1116-7
Hexoprenaline
Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat (Lipshitz et al). 1986; **154**:310-4
Effect of long-term administration of β_2-sympathomimetic drug in the diet-restricted pregnant rat model (Lipshitz et al). 1986; **154**:314-7
Histoplasmosis
Meningoencephalitis caused by *Histoplasma capsulatum* complicating pregnancy (McGregor et al). 1986; **154**:925-31
Home glucose monitoring
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Hospitalization
Effects of maternal transport on admission patterns at a tertiary care center (Cowett et al). 1986; **154**:1098-100
A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance (Der Yuen). 1986; **154**:1206-10
Obstetric hospital stays by diagnosis related groups: A community-based analysis (Lagoe). 1986; **154**:873-8
Human papillomavirus; *see* **Papillomavirus**
Hydatidiform mole
Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line (Sarkar et al). 1986; **154**:390-3
Epidemiology of hydatidiform mole during early gestation (Atrash et al). 1986; **154**:906-9
Occurrence of molar pregnancy in patients undergoing elective abortion: Comparison with other clinical presentations (Ho Yuen and Callegari). 1986; **154**:273-6

Hydrops fetalis
Hydrops fetalis and chromosomal trisomies (Landrum et al). 1986; **154**:1114-5
6-Hydroxydopamine
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
5-Hydroxytryptophan
Influence of sequential doses of 5-hydroxytryptophan on prolactin release (Sueldo et al). 1986; **154**:424-7
Hygroma
Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management (Garden et al). 1986; **154**:221-5
Hyperbilirubinemia
Measurements of delta optical density at 450 nm in SS disease (Nelson) (Letter); (Lindsay and Lupo) (Reply). 1986; **154**:1166
Hyperglycemia; *see also* **Diabetes**
Glucose threshold for macrosomia in pregnancy complicated by diabetes (Willman et al). 1986; **154**:470-5
Hyperinsulinemia
Hyperinsulinemia in hyperthecosis of the ovaries (Nagamani et al). 1986; **154**:384-9
Hyperketonemia
Effect of maternal hyperketonemia in hyperglycemic pregnant ewes and their fetuses (Miodovnik et al). 1986; **154**:394-401
Hyperprolactinemia
Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia (Kletzky et al). 1986; **154**:431-5
Hypertension
Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion (Cotton et al). 1986; **154**:1053-9
Gaucher's disease in pregnancy associated with portal hypertension (Mazor et al). 1986; **154**:1119-20
Hypertensive diseases of pregnancy and parity (Gleicher et al). 1986; **154**:1044-9
The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome (Goldkrand and Fuentes). 1986; **154**:792-800
The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy (Strickland et al). 1986; **154**:146-8
Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema (Cotton et al). 1986; **154**:91-3
Severe preeclampsia with persistent oliguria: Management of hemodynamic subsets (Clark et al). 1986; **154**:490-4
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13
Hyperthecosis
Hyperinsulinemia in hyperthecosis of the ovaries (Nagamani et al). 1986; **154**:384-9
Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist (Steingold et al). 1986; **154**:1241-8
Hypoglycemia
Severe hypoglycemia associated with the HELLP syndrome (Mabie and Sibai) (Letter); (Egley) (Reply). 1986; **154**:211-2
Hypokalemia
Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia (Hendricks et al). 1986; **154**:921-3
Hypotension
Profound hypotension complicating magnesium therapy (Bourgeois et al). 1986; **154**:919-20
Hypovolemia
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
Hypoxia; *see also* **Fetal heart rate**
Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep (Bocking et al). 1986; **154**:329-35
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000
The relationship between antepartum fetal heart rate, intrapartum fetal heart rate, and fetal acid-base status (Low et al). 1986; **154**:769-76
Hysterectomy
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8
Cefotetan versus cefoxitin as prophylaxis in hysterectomy (Orr Jr et al). 1986; **154**:960-3 (Symposium)
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6

I

Imaging
Magnetic resonance imaging and placenta previa (Power et al). 1986; **154**:565-9
Immunoglobulin G
Immunoglobulin G subclasses and isoimmunized pregnancy outcome (Taslimi et al). 1986; **154**:1327-32
Impedance plethysmography
Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Goodlin). 1986; **154**:634-5
In utero transfusion
Intravascular transfusion in utero: The percutaneous approach (Berkowitz et al). 1986; **154**:622-3
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
In vitro fertilization; *see* **Fertilization**
Incision
In defense of the vertical uterine incision (Goodlin) (Letter); (Jovanovic) (Reply). 1986; **154**:966-7
Finger extension of transverse uterine incision (Gerace) (Letter); (Jovanovic) (Reply). 1986; **154**:1168-9
Incompetent cervix
Ultrasound differentiation of the competent from the incompetent cervix: Prevention of preterm delivery (Michaels et al). 1986; **154**:537-46
Incontinence
Modified retropubic cystourethropexy (Goodno Jr and Powers). 1986; **154**:1211-5
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Indomethacin
Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit (Pride et al). 1986; **154**:1155-60

Induced labor
Preinduction cervical ripening with sequential use of prostaglandin E_2 gel (Prins et al). 1986; **154**:1275-9
Infant; *see also* **Neonate**
The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study (Shime et al). 1986; **154**:341-5
Midforceps deliveries: Long-term outcome of infants (Dierker Jr et al). 1986; **154**:764-8
Infection
Cefotetan in the treatment of obstetric and gynecologic infections (Poindexter III et al). 1986; **154**:946-50 (Symposium)
Cefotetan versus cefoxitin as prophylaxis in hysterectomy (Orr Jr et al). 1986; **154**:960-3 (Symposium)
Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section (Varner et al). 1986; **154**:951-4 (Symposium)
Etiology of cervical inflammation (Paavonen et al). 1986; **154**:556-64
Further studies on genital mycoplasmis in intra-amniotic infection: Blood cultures and serologic response (Gibbs et al). 1986; **154**:717-26
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear (Burk et al). 1986; **154**:982-9
Infective endocarditis in obstetric and gynecologic practice (Seaworth and Durack). 1986; **154**:180-8
Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8
Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease (Kiviat et al). 1986; **154**:865-73
Microbial colonization of tailed and tailless intrauterine contraceptive devices: Influence of the mode of insertion in the rabbit (Jacques et al). 1986; **154**:648-55
Mobiluncus-specific antibodies in a postoperative infection (Larsson et al) (Letter). 1986; **154**:1167-8
An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis (Minkoff and Mead). 1986; **154**:973-7
Protease production by microorganisms associated with reproductive tract infection (McGregor et al). 1986; **154**:109-14
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Residues of pelvic inflammatory disease in intrauterine device users: A result of the intrauterine device or *Chlamydia trachomatis* infection? (Guderian and Trobough). 1986; **154**:497-503
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)
Infertility; *see also* **Fertilization**
Analysis of sequential treatment protocols for endometriosis-associated infertility (Olive and Lee). 1986; **154**:613-9
Conservative treatment of ectopic pregnancy with methotrexate (Ory et al). 1986; **154**:1299-1306
Upper genital tract changes and infertility in diethylstilbestrol-exposed women (Kaufman et al). 1986; **154**:1312-8
Inflammation; *see also* **Infection**
Etiology of cervical inflammation (Paavonen et al). 1986; **154**:556-64
Inheritance
Family trait analysis: A case-control study of 43 women with endometriosis and their best friends (Lamb et al). 1986; **154**:596-601
Insemination; *see also* **Embryo transfer; Fertilization**
Experience with intrauterine insemination for cervical factor and oligospermia (Hull et al). 1986; **154**:1333-8
Insulin
The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman (Ray et al). 1986; **154**:1257-63
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Hyperinsulinemia in hyperthecosis of the ovaries (Nagamani et al). 1986; **154**:384-9
Intracranial hemorrhage
Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage (Shankaran et al). 1986; **154**:53-7
Maternal, fetal, and newborn complications associated with newborn intracranial hemorrhage (Low et al). 1986; **154**:345-51
Intraepithelial neoplasia
Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods (Falcone and Ferenczy). 1986; **154**:260-4
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases (Deigen et al). 1986; **154**:255-9
Intrauterine death; *see* **Mortality**
Intrauterine device
Bacterial flora of the cervix in women using different methods of contraception (Haukkamaa et al). 1986; **154**:520-4
Microbial colonization of tailed and tailless intrauterine contraceptive devices: Influence of the mode of insertion in the rabbit (Jacques et al). 1986; **154**:648-55
Residues of pelvic inflammatory disease in intrauterine device users: A result of the intrauterine device or *Chlamydia trachomatis* infection? (Guderian and Trobough). 1986; **154**:497-503
Intrauterine transfusion
Intravascular transfusion in utero: The percutaneous approach (Berkowitz et al). 1986; **154**:622-3
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
Intussusception
Postoperative intussusception after vaginal vault suspension (Hunter et al). 1986; **154**:633-4
Ionizing radiation
An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels (Jarrell et al). 1986; **154**:306-9

Isoimmunization; *see also* Rh isoimmunization
Immunoglobulin G subclasses and isoimmunized pregnancy outcome (Taslimi et al). 1986; **154**:1327-32
Isoproterenol
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5
Items
Items. 1986; **154**:220, 476, 697-8, 972, 1172, 1371-91

J

Jehovah's Witnesses
Blood transfusions and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology (Sacks and Koppes). 1986; **154**:483-6 (Clin. Opinion)
Jet air travel
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000

K

Kegel exercise
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Kell sensitization
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1986; **154**:85-90
Ketone
Effect of maternal hyperketonemia in hyperglycemic pregnant ewes and their fetuses (Miodovnik et al). 1986; **154**:394-401
17-Ketoreductase
Male pseudohermaphroditism due to 17-ketoreductase deficiency: Report of a case without gynecomastia and without vaginal pouch (Caufriez). 1986; **154**:148-9
Kidney
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Prenatal diagnosis of fetal renal mesoblastic nephroma (Apuzzio et al). 1986; **154**:636-7
Septic shock with renal failure after chorionic villus sampling (Barela et al). 1986; **154**:1100-2
Kleihauer-Betke test
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9

L

Labor; *see also* Delivery
Abdominal rescue after entrapment of the aftercoming head (Iffy et al). 1986; **154**:623-4
The effects of oral administration of progesterone for premature labor (Erny et al). 1986; **154**:525-9
Preinduction cervical ripening with sequential use of prostaglandin E_2 gel (Prins et al). 1986; **154**:1275-9
Preterm labor: Its diagnosis and management (Gonik and Creasy). 1986; **154**:3-8 (Clin. Opinion)
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Lactate
Uterine metabolism of the pregnant rabbit under chronic steady-state conditions (Johnson et al). 1986; **154**:1146-51
Lactation; *see* Breast-feeding
Lactogen
Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep (Newnham et al). 1986; **154**:663-6
Langerhans' cell
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Laparoscopy
Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization (Luber et al). 1986; **154**:1264-70
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4
Laparotomy
CA 125 surveillance and second-look laparotomy in ovarian carcinoma (Atack et al). 1986; **154**:287-9
Staging laparotomy in early epithelial ovarian carcinoma (Helewa et al). 1986; **154**:282-6
Laser
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Lecithin/sphingomyelin ratio; *see also* Respiratory distress syndrome
Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios (Hobson et al). 1986; **154**:1023-6
Legal issues
Blood transfusions and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology (Sacks and Koppes). 1986; **154**:483-6 (Clin. Opinion)
Letters
Letters. 1986; **154**:211-7, 689-95, 964-70, 1164-70
Leukocyte-rich erythrocytes
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20
Liability
The Golden Thread (Malkasian Jr). 1986; **154**:1285-90 (Pres. address)
Lipoprotein
The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells (Moise et al). 1986; **154**:1080-5
Lung; *see also* Fetal lung maturity; Respiratory distress syndrome
Effect of induced oligohydramnios on fetal lung development (King et al). 1986; **154**:823-30
Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Goodlin). 1986; **154**:634-5
Pulmonary responses to exercise in pregnancy (Artal et al). 1986; **154**:378-83
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Lupus anticoagulant
Lupus anticoagulant and pregnancy (Spitz et al) (Letter). 1986; **154**:1169

Luteinizing hormone
Histologic and hormonal documentation of the luteinized unruptured follicle syndrome (Schenken et al). 1986; **154**:839-47
Inhibition of gonadotropin-releasing hormone receptors in rat anterior pituitary monolayer cell cultures by danazol (Menon et al). 1986; **154**:362-72
Modulation of luteinizing hormone immunoreactivity and bioactivity by dopamine but not norepinephrine in women (Barnes et al). 1986; **154**:445-50

Luteotropic hormone
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63

Lymphangiography
The role of lymphangiography in vulvar carcinoma (Weiner et al). 1986; **154**:1073-5

M

Macrophage
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay (Chacho et al). 1986; **154**:1290-9

Macrosomia; *see also* **Diabetes**
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Glucose threshold for macrosomia in pregnancy complicated by diabetes (Willman et al). 1986; **154**:470-5

Magnesium sulfate
Profound hypotension complicating magnesium therapy (Bourgeois et al). 1986; **154**:919-20

Magnetic resonance imaging
Magnetic resonance imaging and placenta previa (Power et al). 1986; **154**:565-9

Male pseudohermaphroditism
Male pseudohermaphroditism due to 17-ketoreductase deficiency: Report of a case without gynecomastia and without vaginal pouch (Caufriez). 1986; **154**:148-9

Malignancy
Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus (Chambers and Unis). 1986; **154**:1194-9

Media
An evaluation of different media for the zona-free hamster egg penetration test (Mortimer). 1986; **154**:351-4

Medroxyprogesterone acetate
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:455-61

α-Melanocyte–stimulating hormone level
Fetal α-melanocyte–stimulating hormone levels: No correlation with late fetal growth but increased with diabetes mellitus (Hassan et al). 1986; **154**:428-30

Membranes
The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with premature rupture of membranes (Morales et al). 1986; **154**:591-5
Effect of induced oligohydramnios on fetal lung development (King et al). 1986; **154**:823-30
A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes (Arias et al). 1986; **154**:1059-63

Meningoencephalitis
Meningoencephalitis caused by *Histoplasma capsulatum* complicating pregnancy (McGregor et al). 1986; **154**:925-31

Menopause
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:456-61
Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods (Notelovitz et al). 1986; **154**:1009-13
Estrogen use and cardiovascular disease (Henderson et al). 1986; **154**:1181-6
Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus (Chambers and Unis). 1986; **154**:1194-9

Menstrual cycle
Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle (Pansini et al). 1986; **154**:679-82
Variability and selectivity of anterior pituitary response to dopamine agonists throughout the normal menstrual cycle (Kletzky and Shangold). 1986; **154**:362-7

Mesoblastic nephroma
Prenatal diagnosis of fetal renal mesoblastic nephroma (Apuzzio et al). 1986; **154**:636-7

Metabolism
Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9
Uterine metabolism of the pregnant rabbit under chronic steady-state conditions (Johnson et al). 1986; **154**:1146-51

Metastasis
Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites (Lancaster et al). 1986; **154**:115-9
Staging laparotomy in early epithelial ovarian carcinoma (Helewa et al). 1986; **154**:282-6

Methotrexate
Conservative treatment of ectopic pregnancy with methotrexate (Ory et al). 1986; **154**:1299-1306

Metronidazole
Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8

Microcirculation
Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus (Lee and Yeh). 1986; **154**:1133-9
Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis (Lee and Yeh). 1096; **154**:1139-46

Microsurgery
Microsurgical reversal of sterilization: A six-year study (Spivak et al). 1986; **154**:355-61

Midforceps
Midforceps deliveries: Long-term outcome of infants (Dierker Jr et al). 1986; **154**:764-8
Reply to Dr. Emanuel Friedman (Kadar) (Letter). 1986; **154**:694-5

Midwifery
The perinatal and economic impact of prenatal care in a low-socioeconomic population (Moore et al). 1986;, **154**:29-33

Milk; *see also* Breast-feeding
Excretion of varicella–herpes zoster virus in breast milk (Frederick et al). 1986; **154**:1116-7
M-mode echocardiography
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3
Mobiluncus
Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8
Mobiluncus-specific antibodies in a postoperative infection (Larsson et al) (Letter). 1986; **154**:1167-8
Mole; *see* Hydatidiform mole
Monitoring
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**: 250-4
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Morality; *see* Ethics
Mortality
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**: 235-9
Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death (Erskine et al). 1096; **154**:1109-10
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**: 250-4
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Mucus
The tumor marker CA 125 is a common constituent of normal cervical mucus (de Bruijn et al). 1986; **154**:1088-91
Multicystic kidney disease
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Mycoplasma
Further studies on genital mycoplasmas in intra-amniotic infection: Blood cultures and serologic response (Gibbs et al). 1986; **154**:717-26
Myometrium
α-Adrenergic receptors in human myometrium during pregnancy (Berg et al). 1986; **154**:601-6
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5
Myosin
Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7

N

Nalbuphine hydrochloride
Sinusoidal fetal heart rate pattern after administration of nalbuphine hydrochloride: A case report (Feinstein et al). 1986; **154**:159-60
Naloxone
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Neonate
Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage (Shankaran et al). 1986; **154**: 53-7
Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura (Meschengieser and Lazzari) (Letter); (Martin Jr) (Reply). 1986; **154**:1166-7
Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings (Page et al). 1986; **154**:1306-11
Effects of maternal transport on admission patterns at a tertiary care center (Cowett et al). 1986; **154**:1098-100
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**:250-4
Gestational diabetes: Impact of home glucose monitoring on neonatal birth weight (Goldberg et al). 1986; **154**:546-50
Immunoglobulin G subclasses and isoimmunized pregnancy outcome (Taslimi et al). 1986; **154**:1327-32
Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant (Ostrea Jr et al). 1986; **154**:1014-7
Maternal, fetal, and newborn complications associated with newborn intracranial hemorrhage (Low et al). 1986; **154**:345-51
Neonatal alloimmune thrombocytopenic purpura: A case report (Deaver et al). 1986; **154**:153-5
An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis (Minkoff and Mead). 1986; **154**:973-7
Perinatal events and cerebral palsy (Hensleigh et al). 1986; **154**:978-81
Relative placental size in growth-retarded infants (Woods) (Letter); (Teasdale) (Reply). 1986; **154** 1170
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74
Neoplasia; *see also* Carcinoma
Cervical intraepithelial neoplasia and condyloma: An analysis of diagnostic accuracy of posttreatment follow-up methods (Falcone and Ferenczy). 1986; **154**:260-4
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Testosterone levels and androgen-producing neoplasms (Meldrum) (Letter); (Friedman) (Reply). 1986; **154**: 965-6
Treatment of cervical intraepithelial neoplasia with electrocautery: A report of 776 cases (Deigen et al). 1986; **154**:255-9
Nephroma
Prenatal diagnosis of fetal renal mesoblastic nephroma (Apuzzio et al). 1986; **154**:636-7
Neutropenia
Ritodrine-induced neutropenia (Wang-Cheng and Davidson). 1986; **154**:924-5
Newborn infant; *see* Neonate
Nifedipine
The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat (Veille et al). 1986; **154**:1160-3
Nitrendipine
Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7

Nitroglycerin
Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion (Cotton et al). 1986; **154**:1053-9
Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema (Cotton et al). 1986; **154**:91-3
Norepinephrine
Modulation of luteinizing hormone immunoreactivity and bioactivity by dopamine but not norepinephrine in women (Barnes et al). 1986; **154**:445-50
Plasma catecholamine responses to physiologic stimuli in normal human pregnancy (Barron et al). 1986; **154**:80-4
Nubain; *see* **Nalbuphine hydrochloride**

O

Obesity
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Prepregnancy weight, weight gain, and birth weight (Abrams and Laros Jr). 1986; **154**:503-9
Obstetric outcome
An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries (Andrews et al). 1986; **154**:848-54
An obstetric assessment of the first 100 births from the in vitro fertilization program at Clamart, France (Frydman et al). 1986; **154**:550-5
Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios (Bastide et al). 1986; **154**:895-900
Oligohydramnios
Effect of induced oligohydramnios on fetal lung development (King et al). 1986; **154**:823-30
Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios (Bastide et al). 1986; **154**:895-900
Oligospermia
Experience with intrauterine insemination for cervical factor and oligospermia (Hull et al). 1986; **154**:1333-8
Oliguria
Severe preeclampsia with persistent oliguria: Management of hemodynamic subsets (Clark et al). 1986; **154**:490-4
Omental graft
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6
Oncogene
Demonstration of myc and ras oncogene expression by hybridization in situ in hydatidiform mole and in the BeWo choriocarcinoma cell line (Sarkar et al). 1986; **154**:390-3
Oocyte
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4
Opioid
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Oral contraceptive; *see also* **specific agent**
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Ovary
An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels (Jarrell et al). 1986; **154**:306-9
CA 125 surveillance and second-look laparotomy in ovarian carcinoma (Atack et al). 1986; **154**:287-9
A comparison of three techniques for ovarian reconstruction (Oelsner et al). 1986; **154**:569-72
Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings (el Tabbakh et al). 1986; **154**: 892-5
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Hyperinsulinemia in hyperthecosis of the ovaries (Nagamani et al). 1986; **154**:384-9
Ovarian cancer in the elderly: An analysis of surveillance, epidemiology, and end results program data (Yancik et al). 1986; **154**:639-47 (Curr. Dev.)
Ovarian carcinoma of low malignant potential: Staging and treatment (Nation and Krepart). 1986; **154**:290-3
Ovarian pregnancy with a contralateral corpus luteum: Case report (Check and Chase). 1986; **154**:155-6
Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit (Pride et al). 1986; **154**:1155-60
Staging laparotomy in early epithelial ovarian carcinoma (Helewa et al). 1986; **154**:282-6
Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist (Steingold et al). 1986; **154**:1241-8
The tumor marker CA 125 is a common constituent of normal cervical mucus (de Bruijn et al). 1986; **154**:1088-91
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4
Ovulation
Histologic and hormonal documentation of the luteinized unruptured follicle syndrome (Schenken et al). 1986; **154**:839-47
Oxygen; *see also* **Hypoxia**
Fetal responses to maternal infusions of angiotensin II (Perales et al). 1986; **154**:195-203
Heat flux and oxygen consumption of the pregnant uterus (Rudelstorfer et al). 1986; **154**:462-70
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8
Oxytocin
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63

P

Pacific Coast Obstetrical and Gynecological Society
Transactions of the fifty-second annual meeting of the Pacific Coast Obstetrical and Gynecological Society. 1986; **154**:1173-1284

Pain
Endometriosis in association with uterine anomaly (Sanfilippo et al). 1986; **154**:39-43
Pancytopenia
Folate deficiency presenting as pancytopenia in pregnancy (Solano Jr and Councell). 1986; **154**:1117-8
Papanicolaou smear
An analysis of the factors involved in the colposcopic evaluation of 2194 patients with abnormal Papanicolaou smears (Wetrich). 1986; **154**:1339-49
Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear (Burk et al). 1986; **154**:982-9
Papers
Papers of the Society for Gynecologic Investigation. 1986; **154**:362-475
Papillomavirus
Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites (Lancaster et al). 1986; **154**:115-9
Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: Correlation with biopsy results and Papanicolaou smear (Burk et al). 1986; **154**:982-9
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15
Paracrine regulator
Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis (Ono et al). 1986; **154**:709-16
Parity
The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy (Strickland et al). 1986; **154**:146-8
Parlodel; *see* **Bromocriptine**
Parturition; *see* **Delivery**
Peer review
A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance (Der Yuen). 1986; **154**:1206-10
Pelvic infection; *see* **Infection; Pelvic inflammatory disease**
Pelvic inflammatory disease
Cefotetan in the treatment of obstetric and gynecologic infections (Poindexter III et al). 1986; **154**:946-50 (Symposium)
Localization of *Chlamydia trachomatis* infection by direct immunofluorescence and culture in pelvic inflammatory disease (Kiviat et al). 1986; **154**:865-73
Residues of pelvic inflammatory disease in intrauterine device users: A result of the intrauterine device or *Chlamydia trachomatis* infection? (Guderian and Trobough). 1986; **154**:497-503
Results of microsurgical treatment of tubal infertility and early second-look laparoscopy in the post–pelvic inflammatory disease patient: Implications for in vitro fertilization (Luber et al). 1986; **154**:1264-70
Serum C-reactive protein determination in acute pelvic inflammatory disease (Lehtinen et al). 1986; **154**:158-9
Penis
Colposcopy in the diagnosis of penile condyloma (Sedlacek et al). 1986; **154**:494-6
Pentobarbital
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8
Percutaneous umbilical sampling
Percutaneous umbilical sampling in immune thrombocytopenic purpura (Sacks) (Letter); (Hobbins et al) (Reply). 1986; **154**:214-5
Pergolide
Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia (Kletzky et al). 1986; **154**:431-5
Pericarditis
Constrictive pericarditis and pregnancy (Sachs et al). 1986; **154**:156-7
Perinatal morbidity
Perinatal events and cerebral palsy (Hensleigh et al). 1986; **154**:978-81
Peritoneal fluid
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay (Chacho et al). 1986; **154**:1290-9
Pharmacologic sypathectomy
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
Phenobarbital
Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage (Shankaran et al). 1986; **154**:53-7
Phosphatidylcholine phosphorus and phosphatidylglycerol
Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity (Kogon et al). 1986; **154**:226-30
Effect of aminophylline on lung maturation in preterm rabbit fetuses (Cosmi et al). 1986; **154**:436-9
Phospholipid
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74
Pituitary
Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion (Blumenfeld et al). 1986; **154**:606-12
Inhibition of gonadotropin-releasing hormone receptors in rat anterior pituitary monolayer cell cultures by danazol (Menon et al). 1986; **154**:362-72
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Variability and selectivity of anterior pituitary response to dopamine agonists throughout the normal menstrual cycle (Kletzky and Shangold). 1986; **154**:362-7
Placenta
Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep (Newnham et al). 1986; **154**:663-6
Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat (Lipshitz et al). 1986; **154**:310-4
Effect of long-term administration of β_2-sympathomimetic drug in the diet-restricted pregnant rat model (Lipshitz et al). 1986; **154**:314-7
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74

Fetal microcirculation of abnormal human placenta. I. Scanning electron microscopy of placental vascular casts from small for gestational age fetus (Lee and Yeh). 1986; **154**:1133-9

Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis (Lee and Yeh). 1986; **154**:1139-46

Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9

Human fallopian tube contains placental protein 14 (Julkunen et al). 1986; **154**:1076-9

Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy (Dorko and Hayashi). 1986; **154**:801-5

Maternal ovine placental vascular responses to adenosine (Landauer et al). 1986; **154**:1152-5

Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7

Placental prostacyclin production in normal and toxemic pregnancies (Jeremy et al) (Letter); (Walsh) (Reply). 1986; **154**:212-4

Relative placental size in growth-retarded infants (Woods) (Letter); (Teasdale) (Reply). 1986; **154**:1170

Placenta previa

Magnetic resonance imaging and placenta previa (Power et al). 1986; **154**:565-9

Plasma

Partial purification of relaxin from human seminal plasma (Weiss et al). 1986; **154**:749-55

Plasma catecholamine responses to physiologic stimuli in normal human pregnancy (Barron et al). 1986; **154**:80-4

Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3

Plasma immunoreactive β-endorphin in exercise-associated amenorrhea (Laatikainen et al). 1986; **154**:94-7

Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic (Lazarchick et al). 1986; **154**:1050-2

Sodium excretion in human pregnancy: A role for arginine vasopressin (Brown and Gallery). 1986; **154**:914-9

Platelet

Basal and forskolin-stimulated cyclic adenosine monophosphate in intact human platelets during the menstrual cycle (Pansini et al). 1986; **154**:679-82

An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody (Hart et al). 1986; **154**:878-83

Human platelet α-adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit (Roberts et al). 1986; **154**:206-10

Longitudinal study of platelet indices during normal pregnancy (Tygart et al). 1986; **154**:883-7

Plethysmography

Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Gocdlin). 1986; **154**:634-5

Pneumomediastinum

Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9

Pneumothorax

Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9

Polycystic ovary

Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings (el Tabbakh et al). 1986; **154**:892-5

Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44

Polyhydramnios

Ultrasound evaluation of polyhydramnios and twin pregnancy (Hashimoto et al). 1986; **154**:1069-72

Portal hypertension

Gaucher's disease in pregnancy associated with portal hypertension (Mazor et al). 1986; **154**:1119-20

Postdate pregnancy; *see* **Postmaturity**

Postmaturity

Biophysical profile scoring in the management of the postterm pregnancy: An analysis of 307 patients (Johnson et al). 1986; **154**:269-73

Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9

The influence of prolonged pregnancy on infant development at one and two years of age: A prospective controlled study (Shime et al). 1986; **154**:341-5

Management of postdate pregnancy (Lagrew and Freeman). 1986; **154**:8-13 (Clin. Opinion)

Postmenopausal period; *see also* **Menopause**

Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus (Chambers and Unis). 1986; **154**:1194-9

Postoperative complication

Mobiluncus-specific antibodies in a postoperative infection (Larsson et al) (Letter). 1986; **154**:1167-8

Postoperative intussusception after vaginal vault suspension (Hunter et al). 1986; **154**:633-4

Postpartum mood

Postpartum moods in men and women (Quadagno et al). 1986; **154**:1018-23

Postterm pregnancy; *see* **Postmaturity**

Potassium

Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia (Hendricks et al). 1096; **154**:921-3

Preeclampsia; *see also* **Eclampsia**

Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic (Lazarchick et al). 1986; **154**:1050-2

Profound hypotension complicating magnesium therapy (Bourgeois et al). 1986; **154**:919-20

The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome (Goldkrand and Fuentes). 1986; **154**:792-800

Relative placental size in growth-retarded infants (Woods) (Letter); (Teasdale) (Reply). 1986; **154**:1170

Severe preeclampsia with persistent oliguria: Management of hemodynamic subsets (Clark et al). 1986; **154**:490-4

Pregnancy

α-Adrenergic receptors in human myometrium during pregnancy (Berg et al). 1986; **154**:601-6

Amniotic fluid collagenase inhibitor: Correlation with ges-

tational age and fetal lung maturity (Stricklin et al). 1986; **154**:134-9
Amniotic fluid ferning in early gestation (Borten and Friedman). 1986; **154**:628-30
An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries (Andrews et al). 1986; **154**:848-54
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Antenatal phenobarbital for the prevention of neonatal intracerebral hemorrhage (Shankaran et al). 1986; **154**: 53-7
Aortic function during normal human pregnancy (Hart et al). 1986; **154**:887-91
Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery (Katz et al). 1986; **154**:44-7
Behavior of the squamocolumnar junction during normal pregnancy (Ganesh et al) (Letter). 1986; **154**:689-90
Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep (Newnham et al). 1986; **154**: 663-6
Caffeine consumption during pregnancy and association with late spontaneous abortion (Srisuphan and Bracken). 1986; **154**:14-20
Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion (Cotton et al). 1986; **154**:1053-9
Chorionic villus sampling in continuing pregnancies. I. Low fetal loss rates in initial 109 cases (Elias et al). 1986; **154**:1349-52
Chorionic villus sampling in continuing pregnancies. II. Cytogenetic reliability (Martin et al). 1986; **154**:1353-62
Conservative treatment of ectopic pregnancy with methotrexate (Ory et al). 1986; **154**:1299-1306
Constrictive pericarditis and pregnancy (Sachs et al). 1986; **154**:156-7
Coronary artery disease in diabetic pregnancies (Reece et al). 1986; **154**:150-1
The current role of daily serum estriol monitoring in the insulin-dependent pregnant diabetic woman (Ray et al). 1986; **154**:1257-63
Cushing's syndrome in pregnancy: A case report and literature review (Koerten et al). 1986; **154**:626-8
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat (Veille et al). 1986; **154**:1160-3
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody (Hart et al). 1986; **154**:878-83
Epidemiology of hydatidiform mole during early gestation (Atrash et al). 1986; **154**:906-9
Factors influencing nonstress test results in multiple gestations (Patkos et al). 1986; **154**:1107-8
Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management (Garden et al). 1986; **154**:221-5
Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9
Folate deficiency presenting as pancytopenia in pregnancy (Solano Jr and Councell). 1986; **154**:1117-8
Gaucher's disease in pregnancy associated with portal hypertension (Mazor et al). 1986; **154**:1119-20
Goiter in pregnancy (Hegedüs et al) (Letter); (Long et al) (Reply). 1986; **154**:1164
Heat flux and oxygen consumption of the pregnant uterus (Rudelstorfer et al). 1986; **154**:462-70
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Human chorionic gonadotropin in early normal and pathologic pregnancy (Steier et al). 1986; **154**:1091-4
Human platelet α-adrenergic receptors and responses during pregnancy: No change except that with differing hematocrit (Roberts et al). 1986; **154**:206-10
Hypertensive diseases of pregnancy and parity (Gleicher et al). 1986; **154**:1044-9
Immunoglobulin G subclasses and isoimmunized pregnancy outcome (Taslimi et al). 1986; **154**:1327-32
Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy (Dorko and Hayashi). 1986; **154**:801-5
Infective endocarditis in obstetric and gynecologic practice (Seaworth and Durack). 1986; **154**:180-8
Interrelationship between amniotic fluid C-peptide and catecholamines in the last trimester of diabetic pregnancy (Pschera et al). 1986; **154**:48-52
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1986; **154**:85-90
Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Goodlin). 1986; **154**:634-5
Longitudinal study of platelet indices during normal pregnancy (Tygart et al). 1986; **154**:883-7
Lupus anticoagulant and pregnancy (Spitz et al) (Letter). 1986; **154**:1169
Management of postdate pregnancy (Lagrew and Freeman). 1986; **154**:8-13 (Clin. Opinion)
Maternal and fetal surveillance for older pregnant women (Schipul Jr et al) (Letter); (Kirz) (Reply). 1986; **154**: 967-8
Maternal ovine placental vascular responses to adenosine (Landauer et al). 1986; **154**:1152-5
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9
Meningoencephalitis caused by *Histoplasmc capsulatum* complicating pregnancy (McGregor et al). 1986; **154**:925-31
Multiple peripheral pulmonic stenosis in pregnancy (Landsberger and Grossman III). 1986; **154**:152-3
Ovarian pregnancy with a contralateral corpus luteum: Case report (Check and Chase). 1986; **154**:155-6
Perforated adenocarcinoma of the appendix during pregnancy (Donnenfeld et al). 1986; **154**:637-8
The perinatal and economic impact of prenatal care in a low-socioeconomic population (Moore et al). 1986; **154**:29-33
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000
Placental prostacyclin production in normal and toxemic pregnancies (Jeremy et al) (Letter); (Walsh) (Reply). 1986; **154**:212-4

Plasma catecholamine responses to physiologic stimuli in normal human pregnancy (Barron et al). 1986; **154**:80-4
Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic (Lazarchick et al). 1986; **154**:1050-2
Prepregnancy weight, weight gain, and birth weight (Abrams and Laros Jr). 1986; **154**:503-9
Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department (Malecki et al). 1986; **154**:625-6
The relation of angiotensin-converting enzyme to the pregnancy-induced hypertension–preeclampsia syndrome (Goldkrand and Fuentes). 1986; **154**:792-800
The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy (Strickland et al). 1986; **154**:146-8
Relationship between log of the human chorionic gonadotropin concentration and time period in early pregnancy (Kadar) (Letter); (Pittaway) (Reply). 1986; **154**:692-4
Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon (Camiel). 1986; **154**:1104-5
Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema (Cotton et al). 1986; **154**:91-3
Serum fructosamine: A screening test for diabetes in pregnancy (Roberts and Baker). 1986; **154**:1027-30
Should a fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? (Coustan et al) 1986; **154**:1031-5
Sodium excretion in human pregnancy: A role for arginine vasopressin (Brown and Gallery). 1986; **154**:914-9
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6
Tubal conservation in ectopic pregnancy: A study of 200 cases (Hallatt). 1986; **154**:1216-21
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3
Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios (Bastide et al). 1986; **154**:895-900
Ultrasound evaluation of polyhydramnios and twin pregnancy (Hashimoto et al). 1986; **154**:1069-72
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9
Uterine activity during pregnancy in ambulatory patients: Comparison of singleton and twin gestations (Newman et al). 1986; **154**:530-1
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13
Uterine metabolism of the pregnant rabbit under chronic steady-state conditions (Johnson et al). 1986; **154**:1146-51

Premature labor
The effects of oral administration of progesterone for premature labor (Erny et al). 1986; **154**:525-9

Premature rupture of membranes; *see* **Membranes**

Prematurity; *see also* **Preterm labor and delivery**
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**:250-4
The perinatal and economic impact of prenatal care in a low-socioeconomic population (Moore et al). 1986; **154**:29-33
Plasma growth hormone concentration in the chronically catheterized ovine fetus during spontaneous term delivery and premature delivery induced by continuous intravascular infusion of low doses of adrenocorticotropin or cortisol to the fetus (Lowe et al). 1986; **154**:420-3

Premenstrual syndrome
A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome (Maddocks et al). 1986; **154**:573-81

Prenatal care
The perinatal and economic impact of prenatal care in a low-socioeconomic population (Moore et al). 1986; **154**:29-33
Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department (Malecki et al). 1986; **154**:625-6

Presentation; *see* **Breech presentation**

Presidential address
Presidential address. 1986; **154**:699-708, 1173-80, 1285-90

Preterm labor and delivery
Adjunctive erythomycin treatment for idiopathic preterm labor: Results of a randomized, double-blinded, placebo-controlled trial (McGregor et al). 1986; **154**:98-103
Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery (Katz et al). 1986; **154**:44-7
Detection of preterm labor by ambulatory monitoring of uterine activity for the management of oral tocolysis (Katz et al). 1986; **154**:1253-6
Effect of aminophylline on lung maturation in preterm rabbit fetuses (Cosmi et al). 1986; **154**:436-9
Failure to demostrate decreased β-adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition (Dattel et al). 1986; **154**:450-6
Fetal heart rate monitoring and neonatal mortality in the very preterm infant (Braithwaite et al). 1986; **154**:250-4
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Preterm labor: Its diagnosis and management (Gonik and Creasy). 1986; **154**:3-8 (Clin. Opinion)
A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes (Arias et al). 1986; **154**:1059-63
Ultrasound differentiation of the competent from the incompetent cervix: Prevention of preterm delivery (Michaels et al). 1986; **154**:537-46

Progesterone; *see also* **Progestin**
A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome (Maddocks et al). 1986; **154**:573-81
The effect of dehydroepiandrosterone sulfate on de novo and low-density lipoprotein–stimulated progesterone secretion by human choriocarcinoma JEG-3 cells (Moise et al). 1986; **154**:1080-5
The effects of oral administration of progesterone for premature labor (Erny et al). 1986; **154**:525-9
Fibrocystic breast disease: Pathophysiology, pathomorphology, clinical picture, and management (Vorherr). 1986; **154**:161-79
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63

Moderate recreational running is associated with lowered salivary progesterone profiles in women (Ellison and Lager). 1986; **154**:1000-3

Progestin; *see also* **Progesterone**
Biochemical and histologic effects of sequential estrogen/progestin therapy on the endometrium of postmenopausal women (Gibbons et al). 1986; **154**:456-61
Histologic and hormonal documentation of the luteinized unruptured follicle syndrome (Schenken et al). 1986; **154**:839-47

Prolactin
Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings (el Tabbakh et al). 1986; **154**: 892-5
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
Epithelial regulation of prolactin effect on amnionic permeability (Raabe and McCoshen). 1986; **154**:130-4
Influence of sequential doses of 5-hydroxytryptophan on prolactin release (Sueldo et al). 1986; **154**:424-7
Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast (Ho Yuen et al). 1986; **154**:336-40
Opioid regulation of pituitary gonadotropins and prolactin in women using oral contraceptives (Snowden et al). 1986; **154**:440-4
Pergolide and bromocriptine for the treatment of patients with hyperprolactinemia (Kletzky et al). 1096; **154**: 431-5

Prolonged pregnancy; *see* **Postmaturity**

Prostacyclin
Placental prostacyclin production in normal and toxemic pregnancies (Jeremy et al) (Letter); (Walsh) (Reply). 1986; **154**:212-4

Prostaglandin
Preinduction cervical ripening with sequential use of prostaglandin E_2 gel (Prins et al). 1986; **154**:1275-9
Prostaglandin secretion by adrenal tissue of human anencephalic fetuses (Carr et al). 1986; **154**:373-8
Prostaglandins and endometriosis (Sgarlata and Hertelendy) (Letter); (James) (Reply). 1986; **154**:964-5
Relationship of gonadotropin-releasing hormone, danazol, and prostaglandin blockade to ovarian enlargement and ascites formation of the ovarian hyperstimulation syndrome in the rabbit (Pride et al). 1986; **154**:1155-60

Prostanoid
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay (Chacho et al). 1986; **154**:1290-9

Protease
Protease production by microorganisms associated with reproductive tract infection (McGregor et al). 1986; **154**: 109-14

Protein
Biochemical and physiologic characterization of follicle regulatory protein: A paracrine regulator of folliculogenesis (Ono et al). 1986; **154**:709-16
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5
Human fallopian tube contains placental protein 14 (Julkunen et al). 1986; **154**:1076-9
Serum C-reactive protein determination in acute pelvic inflammatory disease (Lehtinen et al). 1986; **154**:158-9
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6

Pseudohermaphroditism
Male pseudohermaphroditism due to 17-ketoreductase deficiency: Report of a case without gynecomastia and without vaginal pouch (Caufriez). 1986; **154**:148-9

Publications
Summary of analysis of published articles. 1986; **154**:477-8 (Editorial)

Puerperal fever
Lactation suppression and puerperal fever (Almeida and Kitay). 1986; **154**:940-1

Pulmonary circulation
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6

Pulmonary edema
Leg static pressure values in pregnant women with iatrogenic pulmonary edema (Goodlin). 1986; **154**:634-5
Role of intravenous nitroglycerin in the treatment of severe pregnancy-induced hypertension complicated by pulmonary edema (Cotton et al). 1986; **154**:91-3

Pulmonic stenosis
Multiple peripheral pulmonic stenosis in pregnancy (Landsberger and Grossman III). 1986; **154**:152-3

Purpura; *see* **Thrombocytopenia**

Q

Quality assurance
A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance (Der Yuen). 1986; **154**:1206-10

R

Radiation
An analysis of the effects of increasing doses of ionizing radiation to the exteriorized rat ovary on follicular development, atresia, and serum gonadotropin levels (Jarrell et al). 1986; **154**:306-9
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6

Rape
Reported sexual assault in Nashville–Davidson County, Tennessee, 1980 to 1982 (Cartwright et al). 1986; **154**:1064-8

Reconstruction
A comparison of three techniques for ovarian reconstruction (Oelsner et al). 1986; **154**:569-72

Red blood cell; *see* **Erythrocyte**

Reference material
Proper study and use of reference material (Hein) (Letter); (Gortmaker) (Reply). 1986; **154**:216-7

Relaxin
Partial purification of relaxin from human seminal plasma (Weiss et al). 1986; **154**:749-55
Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon (Camiel). 1986; **154**:1104-5

Renal failure; *see also* **Kidney**
Septic shock with renal failure after chorionic villus sampling (Barela et al). 1986; **154**:1100-2

Reproductive tract abnormality; *see* **Anomaly**

Reproductive tract infection; *see* **Infection**

Residency
Experience with flexibility in an academic residency program (Hendricks). 1986; **154**:780-5 (Symposium)
Flexibility in obstetric-gynecologic residencies: Why is it necessary? (Willson) 1096; **154**:777-8 (Symposium)
The Golden Thread (Malkasian Jr). 1986; **154**:1285-90 (Pres. address)
The need for flexibility in preparing clinician-scientists for academic careers (Jaffe). 1986; **154**:778-80 (Symposium)
Response on the need for flexibility in academic obstetric-gynecologic residencies (Merrill). 1986; **154**:785-8 (Symposium)
Response on the need for flexibility in academic obstetric-gynecologic residencies (Pitkin). 1986; **154**:788-90 (Symposium)

Respiratory distress syndrome
Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity (Kogon et al). 1986; **154**:226-30
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
Effect of acetone precipitation on the clinical prediction of respiratory distress syndrome when utilizing amniotic fluid lecithin/sphingomyelin ratios (Hobson et al). 1986; **154**:1023-6
The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with premature rupture of membranes (Morales et al). 1986; **154**:591-5
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74

Respiratory function
Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods (Notelovitz et al). 1986; **154**:1009-13

Retinol
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5

Retropubic cystourethropexy
Modified retropubic cystourethropexy (Goodno Jr and Powers). 1986; **154**:1211-5

Rh isoimmunization
Another view of controversy about Rh prophylaxis (Stephens) (Letter). 1986; **154**:968-9
Fetal microcirculation of abnormal human placenta. II. Scanning electron microscopy of placental vascular casts from fetus with severe erythroblastosis fetalis (Lee and Yeh). 1986; **154**:1139-46
Immunoglobulin G subclasses and isoimmunized pregnancy outcome (Taslimi et al). 1986; **154**:1327-32
Intravascular transfusion in utero: The percutaneous approach (Berkowitz et al). 1986; **154**:622-3
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1096; **154**:85-90
Maternal-fetal ABO/Rh antigenic relationships and human fetal development (Hoff and Bixler). 1986; **154**:126-9
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9

Ribonucleic acid
Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy (Dorko and Hayashi). 1986; **154**:801-5

Risk
Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery (Katz et al). 1986; **154**:44-7
Has use of cesarean section reduced the risks of delivery in the preterm breech presentation? (Bodmer et al). 1986; **154**:244-50
Risk factors for the development of diethylstilbestrol-associated clear cell adenocarcinoma: A case-control study (Herbst et al). 1986; **154**:814-22
Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients (McGregor et al). 1986; **154**:955-60 (Symposium)
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74

Risk management
A large community hospital's experience with an obstetrics-gynecology risk management committee: Quality assurance (Der Yuen). 1986; **154**:1206-10

Ritodrine
Effect of ritodrine on uteroplacental blood flow and cardiac output distribution in unanesthetized pregnant guinea pigs (van de Walle and Martin Jr). 1986; **154**:189-94
Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia (Hendricks et al). 1986; **154**:921-3
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5
Ritodrine-induced neutropenia (Wang-Cheng and Davidson). 1986; **154**:924-5

Robin anomalad
The prenatal diagnosis of Robin anomalad (Pilu et al). 1986; **154**:630-2

Rods
Is there a causal connection between motile curved rods, *Mobiluncus* species, and bleeding complications? (Larsson and Bergman). 1986; **154**:107-8

Rosette
Use of the erythrocyte rosette test to screen for excessive fetomaternal hemorrhage in Rh-negative women (Stedman et al). 1986; **154**:1363-9

Roster
Roster of American obstetric and gynecologic societies. 1986; **154**:218-9

Running; *see also* **Exercise**
Moderate recreational running is associated with lowered salivary progesterone profiles in women (Ellison and Lager). 1986; **154**:1000-3

S

Salpingitis; *see* **Infection; Pelvic inflammatory disease**

Semen
Partial purification of relaxin from human seminal plasma (Weiss et al). 1986; **154**:749-55

Sensitization; *see also* **Rh isoimmunization**
Kell sensitization in pregnancy (Caine and Mueller-Heubach). 1986; **154**:85-90

Septic shock
Septic shock with renal failure after chorionic villus sampling (Barela et al). 1986; **154**:1100-2

Serotonin
Influence of sequential doses of 5-hydroxytryptophan on prolactin release (Sueldo et al). 1986; **154**:424-7
Sex
Relationship of baseline fetal heart rate to gestational age and fetal sex (Druzin et al). 1986; **154**:1102-3
Sex hormone
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44
Sexual assault
Reported sexual assault in Nashville–Davidson County, Tennessee, 1980 to 1982 (Cartwright et al). 1986; **154**:1064-8
Shock
Septic shock with renal failure after chorionic villus sampling (Barela et al). 1986; **154**:1100-2
Shoulder dystocia
Another technique for resolution of shoulder dystocia (Carter) (Letter). 1986; **154**:964
Sickle cell anemia
Measurements of delta optical density at 450 nm in SS disease (Nelson) (Letter); (Lindsay and Lupo) (Reply). 1986; **154**:1166
Small bowel obstruction
Disagreement on use of water-soluble contrast material in small bowel obstruction (Ott et al) (Letter); (Robertson) (Reply). 1986; **154**:690-1
Smoking
Cigarette smoking and endometrial cancer (Baron) (Letter). 1986; **154**:965
Societies; *see also* **Papers; Transactions**
Roster of American obstetric and gynecologic societies. 1986; **154**:218-9
Society for Gynecologic Investigation
Papers of the Society for Gynecologic Investigation. 1986; **154**:362-475
Society of Obstetricians and Gynaecologists of Canada
Transactions of the forty-first annual meeting of the Society of Obstetricians and Gynaecologists of Canada. 1986; **154**:221-361
Socioeconomic level
The perinatal and economic impact of prenatal care in a low-socioeconomic population (Moore et al). 1986; **154**:29-33
Prevalence of hepatitis B surface antigen among women receiving prenatal care at the Palm Beach County Health Department (Malecki et al). 1986; **154**:625-6
Sodium
Sodium excretion in human pregnancy: A role for arginine vasopressin (Brown and Gallery). 1986; **154**:914-9
Sodium pentobarbital
Oxygen consumption in fetal lambs after maternal administration of sodium pentobarbital (Rurak and Taylor). 1986; **154**:674-8
Sonography; *see* **Ultrasound**
Spermatozoa
An evaluation of different media for the zona-free hamster egg penetration test (Mortimer). 1986; **154**: 351-4
Peritoneal fluid in patients with and without endometriosis: Prostanoids and macrophages and their effect on the spermatozoa penetration assay (Chacho et al). 1986; **154**:1290-9
Spontaneous abortion; *see* **Abortion**
Squamocolumnar junction
Behavior of the squamocolumnar junction during normal pregnancy (Ganesh et al) (Letter). 1986; **154**:689-90
Squamous cell
Squamous cells in the maternal pulmonary circulation (Clark et al). 1986; **154**:104-6
SS disease
Measurements of delta optical density at 450 nm in SS disease (Nelson) (Letter); (Lindsay and Lupo) (Reply). 1986; **154**:1166
Staging
Ovarian carcinoma of low malignant potential: Staging and treatment (Nation and Krepart). 1986; **154**:290-3
Staging laparotomy in early epithelial ovarian carcinoma (Helewa et al). 1986; **154**:282-6
Staphylococcal toxin
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Sterilization
Microsurgical reversal of sterilization: A six-year study (Spivak et al). 1986; **154**:355-61
Sterilization failures (Stock) (Letter); (Soderstrom) (Reply). 1986; **154**:970
Steroids
Fetoplacental steroid metabolism in prolonged pregnancies (Reynolds et al). 1986; **154**:74-9
Human ovarian oxytocin: Its source and relationship to steroid hormones (Dawood and Khan-Dawood). 1986; **154**:756-63
A retrospective study on the effects of steroid administration and prolongation of the latent phase in patients with preterm premature rupture of the membranes (Arias et al). 1986; **154**:1059-63
Streptococci
An obstetric approach to the prevention of early-onset group B β-hemolytic streptococcal sepsis (Minkoff and Mead). 1986; **154**:973-7
Stress incontinence
Modified retropubic cystourethropexy (Goodno Jr and Powers). 1986; **154**:1211-5
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Subcutaneous emphysema
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Sulfamethoxazole
Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section (Ganesh et al). 1986; **154**:1113-4
Superfusion
Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion (Blumenfeld et al). 1986; **154**:606-12
Surfactant; *see* **Respiratory distress syndrome**
Suture technique
A comparison of three techniques for ovarian reconstruction (Oelsner et al). 1986; **154**:569-72
Sympathectomy
Circulatory responses to hypovolemia in the pregnant and nonpregnant sheep after pharmacologic sympathectomy (Tabsh et al). 1986; **154**:411-9
β_2-Sympathomimetic drug
Effect of long-term administration of β_2-sympathomimetic drug in the diet-restricted pregnant rat model (Lipshitz et al). 1986; **154**:314-7

Symphysis pubis
Relaxin and the radiolucent fissure in the symphysis pubis during pregnancy: The gas phenomenon (Camiel). 1986; **154**:1104-5
Symposium
Symposium on cefotetan. 1986; **154**:945-63
Symposium on the need for flexibility in academic obstetric-gynecologic residencies: Why is it necessary? 1986; **154**:777-91
Syndrome
Amniotic fluid phosphatidylglycerol and phosphatidylcholine phosphorus as predictors of fetal lung maturity (Kogon et al). 1986; **154**:231-4
Cushing's syndrome in pregnancy: A case report and literature review (Koerten et al). 1986; **154**:625-8
Severe hypoglycemia associated with the HELLP syndrome (Mabie and Sibai) (Letter); (Egley) (Reply). 1986; **154**: 211-2
A sonographic sign for fetal Down's syndrome (Defoort and Thiery) (Letter). 1986; **154**:1169-70
Subcutaneous emphysema, pneumomediastinum, and pneumothorax in labor and delivery (Reeder). 1986; **154**:487-9
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Use of the drop volume of amniotic fluid in estimating the risk for respiratory distress syndrome in the newborn infant (Åberg and Gislen). 1986; **154**:68-74

T

Tachycardia
Electrocardiographic changes associated with ritodrine-induced maternal tachycardia and hypokalemia (Hendricks et al). 1986; **154**:921-3
Teratology
The value of case reports in human teratology (Goldberg and Golbus). 1986; **154**:479-82 (Clin. Opinion)
Tertiary care center
Effects of maternal transport on admission patterns at a tertiary care center (Cowett et al). 1986; **154**:1098-100
Testosterone
Elevated concentrations of the β-subunit of human chorionic gonadotropin and testosterone in the amniotic fluid of gestations of diabetic mothers (Barbieri et al). 1986; **154**:1039-43
Testosterone levels and androgen-producing neoplasms (Meldrum) (Letter); (Friedman) (Reply). 1986; **154**: 965-6
Treatment of severe androgen excess due to ovarian hyperthecosis with a long-acting gonadotropin-releasing hormone agonist (Steingold et al). 1986; **154**:1241-8
Thrombocytopenia
Breast-feeding in thrombocytopenic neonates secondary to maternal autoimmune thrombocytopenic purpura (Meschengieser and Lazzari) (Letter); (Martin Jr) (Reply). 1986; **154**:1166-7
An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody (Hart et al). 1986; **154**:878-83
Neonatal alloimmune thrombocytopenic purpura: A case report (Deaver et al). 1986; **154**:153-5
Percutaneous umbilical sampling in immune thrombocytopenic purpura (Sacks) (Letter); (Hobbins et al) (Reply). 1986; **154**:214-5
Thrombosis
Fetomaternal hemorrhage associated with umbilical vein thrombosis (Hoag). 1986; **154**:1271-4
Tinidazole
Are preoperative antibiotics helpful in abdominal hysterectomy? (Senior and Steigrad) 1986; **154**:1004-8
Tocolysis
Detection of preterm labor by ambulatory monitoring of uterine activity for the management of oral tocolysis (Katz et al). 1986; **154**:1253-6
The effects of oral administration of progesterone for premature labor (Erny et al). 1986; **154**:525-9
External cephalic version of the breech presentation under tocolysis (Morrison et al). 1986; **154**:900-3
Prophylactic tocolysis of twins (O'Leary). 1986; **154**:904-5
Ritodrine-induced neutropenia (Wang-Cheng and Davidson). 1986; **154**:924-5
Total abdominal hysterectomy; *see also* **Hysterectomy**
Total abdominal hysterectomy after radiation therapy for cervical cancer: Use of omental graft for fistula prevention (Petty et al). 1986; **154**:1222-6
Toxemia; *see also* **Eclampsia**
Expanded toxemia syndrome or gestosis (Goodlin). 1986; **154**:1227-33
Placental prostacyclin production in normal and toxemic pregnancies (Jeremy et al) (Letter); (Walsh) (Reply). 1986; **154**:212-4
Toxic shock syndrome
Toxic shock syndrome, contraceptive methods, and vaginitis (Lanes et al). 1986; **154**:989-91
Toxic shock syndrome following carbon dioxide laser treatment of genital tract condyloma acuminatum (Bowen et al). 1986; **154**:145-6
Training; *see* **Residency**
Transactions
Transactions of the fifty-third annual meeting of the Central Association of Obstetricians and Gynecologists. 1986; **154**:1285-1369
Transactions of the forty-first annual meeting of the Society of Obstetricians and Gynaecologists of Canada. 1986; **154**:221-361
Transactions of the fourth annual meeting of the American Gynecological and Obstetrical Society. 1986; **154**:699-854
Transactions of the fifty-second annual meeting of the Pacific Coast Obstetrical and Gynecological Society. 1986; **154**:1173-1284
Transfusions
Blood transfusions and Jehovah's Witnesses: Medical and legal issues in obstetrics and gynecology (Sacks and Koppes). 1986; **154**:483-6 (Clin. Opinion)
The effects of fetal exchange transfusion with a red blood cell substitute (Ambrose et al). 1986; **154**:667-74
Intravascular transfusion in utero: The percutaneous approach (Berkowitz et al). 1986; **154**:622-3
Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion (Unander and Lindholm). 1986; **154**:516-20
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
Transport
Effects of maternal transport on admission patterns at a tertiary care center (Cowett et al). 1986; **154**:1098-100
Trauma
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**:235-9

Travel
Physiologic changes in pregnant women and their fetuses during jet air travel (Huch et al). 1986; **154**:996-1000
Trimethoprim-sulfamethoxazole
Single-dose trimethoprim-sulfamethoxazole prophylaxis for cesarean section (Ganesh et al). 1986; **154**:1113-4
Trisomy
Hydrops fetalis and chromosomal trisomies (Landrum et al). 1986; **154**:1114-5
Maternal serum α-fetoprotein and fetal autosomal trisomies (Doran et al). 1986; **154**:277-81
Tritium
Incorporation of labeled ribonucleic acid precursors into maternal and fetal rat tissues during pregnancy (Dorko and Hayashi). 1986; **154**:801-5
Trophoblast
Inhibition of human chorionic gonadotropin production by prolactin from term human trophoblast (Ho Yuen et al). 1986; **154**:336-40
Tumor marker
CA 125 surveillance and second-look laparotomy in ovarian carcinoma (Atack et al). 1986; **154**:287-9
The tumor marker CA 125 is a common constituent of normal cervical mucus (de Bruijn et al). 1986; **154**:1088-91
Tumor markers in ovarian cancer: A comparison between immunosuppressive acidic protein and α_1-acid glycoprotein (Mogensen et al) (Letter). 1986; **154**:1165-6
Twins
Birth asphyxia, trauma, and mortality in twins: Has cesarean section improved outcome? (Bell et al). 1986; **154**:235-9
Cesarean delivery of the second twin after vaginal delivery of the first twin (Rattan et al). 1986; **154**:936-9
Factors influencing nonstress test results in multiple gestations (Patkos et al). 1986; **154**:1107-8
Prophylactic tocolysis of twins (O'Leary). 1986; **154**:904-5
Ultrasound evaluation of polyhydramnios and twin pregnancy (Hashimoto et al). 1986; **154**:1069-72
Uterine activity during pregnancy in ambulatory patients: Comparison of singleton and twin gestations (Newman et al). 1986; **154**:530-1
Two-dimensional echocardiography
Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth (Shime et al). 1986; **154**:294-300
Two-dimensional and M-mode echocardiographic findings in hypertensive pregnant women (Sánchez et al). 1986; **154**:910-3

U

Ultrasound
Antenatal diagnosis of renal anomalies with ultrasound. IV. Bilateral multicystic kidney disease (D'Alton et al). 1986; **154**:532-7
Congenital heart disease and extracardiac anomalies: Associations and indications for fetal echocardiography (Copel et al). 1986; **154**:1121-32 (Curr. Dev.)
Correlation of the ultrasonic appearance of the ovaries in polycystic ovarian disease and the clinical, hormonal, and laparoscopic findings (el Tabbakh et al). 1986; **154**:892-5
Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death (Erskine et al). 1986; **154**:1109-10
Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management (Garden et al). 1986; **154**:221-5
Magnetic resonance imaging and placenta previa (Power et al). 1986; **154**:565-9
A sonographic sign for fetal Down's syndrome (Defoort and Thiery) (Letter). 1986; **154**:1169-70
Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus (Chambers and Unis). 1986; **154**:1194-9
Ultrasound differentiation of the competent from the incompetent cervix: Prevention of preterm delivery (Michaels et al). 1986; **154**:537-46
Ultrasound evaluation of amniotic fluid: Outcome of pregnancies with severe oligohydramnios (Bastide et al). 1986; **154**:895-900
Ultrasound evaluation of polyhydramnios and twin pregnancy (Hashimoto et al). 1986; **154**:1069-72
"Ultrasound rescue": A successful alternative form of oocyte recovery in patients with periovarian adhesions (Taylor et al). 1986; **154**:240-4
Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization (Seeds and Bowes Jr). 1986; **154**:1105-7
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13
Umbilical artery
Blunted response of maternal ovine placental lactogen levels to arginine stimulation after single umbilical artery ligation in pregnant sheep (Newnham et al). 1986; **154**:663-6
The effect of maternal consumption of alcohol on human umbilical artery blood flow (Erskine and Ritchie). 1986; **154**:318-21
Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death (Erskine et al). 1986; **154**:1109-10
Umbilical cord
Decreased fetal cord prolactin concentration in diabetic pregnancies (Saltzman et al). 1986; **154**:1035-8
The length of the human umbilical cord in vertex and breech presentations (Soernes and Bakke). 1986; **154**:1086-7
Percutaneous umbilical sampling in immune thrombocytopenic purpura (Sacks) (Letter); (Hobbins et al) (Reply). 1986; **154**:214-5
Umbilical vein
Fetomaternal hemorrhage associated with umbilical vein thrombosis (Hoag). 1986; **154**:1271-4
Urinary incontinence
Modified retropubic cystourethropexy (Goodno Jr and Powers). 1986; **154**:1211-5
The role of biofeedback in Kegel exercise training for stress urinary incontinence (Burgio et al). 1986; **154**:58-64
Uterine blood flow
The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat (Veille et al). 1986; **154**:1160-3
Effect of ritodrine on uteroplacental blood flow and cardiac output distribution in unanesthetized pregnant guinea pigs (van de Walle and Martin Jr). 1986; **154**:189-94
Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep (Bocking et al). 1986; **154**:329-35
Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep (Bocking and Harding). 1986; **154**:655-62
Extracorporeal perfusion of the human uterus (Bulletti et al). 1986; **154**:683-8

Temporal and receptor correlates of the estrogen response in sheep (Greiss Jr et al). 1986; **154**:831-8
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13

Uterus
Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery (Katz et al). 1986; **154**:44-7
In defense of the vertical uterine incision (Goodlin) (Letter); (Jovanovic) (Reply). 1986; **154**:956-7
Effect of hexoprenaline on uteroplacental blood flow in the pregnant rat (Lipshitz et al). 1985; **154**:310-4
Effects of reduced uterine blood flow on electrocortical activity, breathing, and skeletal muscle activity in fetal sheep (Bocking and Harding). 1986; **154**:655-62
Endometriosis in association with uterine anomaly (Sanfilippo et al). 1986; **154**:39-43
Extracorporeal perfusion of the human uterus (Bulletti et al). 1986; **154**:683-8
Failure to demostrate decreased β-adrenergic receptor concentration or decreased agonist efficacy in term or preterm human parturition (Dattel et al). 1986; **154**:450-6
Finger extension of transverse uterine incision (Gerace) (Letter); (Jovanovic) (Reply). 1986; **154**:1168-9
Heat flux and oxygen consumption of the pregnant uterus (Rudelstorfer et al). 1986; **154**:462-70
Myometrial desensitization to continuous but not to intermittent β-adrenergic agonist infusion in the sheep (Casper and Lye). 1986; **154**:301-5
Pharmacologic levels of nitrendipine do not affect actin-myosin interaction in the human uterus and placenta (Sakamoto and Huszar). 1986; **154**:402-7
Temporal and receptor correlates of the estrogen response in sheep (Greiss Jr et al). 1986; **154**:831-8
Ultrasonographic evidence of uterine malignancy in the postmenopausal uterus (Chambers and Unis). 1986; **154**:1194-9
Uterine activity during pregnancy in ambulatory patients: Comparison of singleton and twin gestations (Newman et al). 1986; **154**:530-1
Uterine metabolism of the pregnant rabbit under chronic steady-state conditions (Johnson et al). 1986; **154**:1146-51

Utilization review
Obstetric hospital stays by diagnosis related groups: A community-based analysis (Lagoe). 1986; **154**:873-8

V

Vagina
Amniostat-FLM: An initial clinical trial with both vaginal pool and amniocentesis samples (Benoit et al). 1986; **154**:65-8
Vaginal colonization with *Escherichia coli* in healthy women (Chow et al). 1986; **154**:120-6

Vaginal suppository
A double-blind placebo-controlled trial of progesterone vaginal suppositories in the treatment of premenstrual syndrome (Maddocks et al). 1986; **154**:573-81

Vaginal vault suspension
Postoperative intussusception after vaginal vault suspension (Hunter et al). 1986; **154**:633-4

Vaginitis
Toxic shock syndrome, contraceptive methods, and vaginitis (Lanes et al). 1986; **154**:989-91

Varicella
Excretion of varicella–herpes zoster virus in breast milk (Frederick et al). 1986; **154**:1116-7

Vasopressin
Sodium excretion in human pregnancy: A role for arginine vasopressin (Brown and Gallery). 1986; **154**:914-9

Velocimetry
Uterine artery Doppler velocimetry in pregnant women with hypertension (Fleischer et al). 1986; **154**:806-13

Version
External cephalic version of the breech presentation under tocolysis (Morrison et al). 1986; **154**:900-3

Vertex presentation
The length of the human umbilical cord in vertex and breech presentations (Soernes and Bakke). 1986; **154**:1086-7

Vertical uterine incision
In defense of the vertical uterine incision (Goodlin) (Letter); (Jovanovic) (Reply). 1986; **154**:966-7

Vibratory acoustic stimulation
Fetal response to vibratory acoustic stimulation in periods of low heart rate reactivity and low activity (Ohel et al). 1986; **154**:619-21

Virus
Excretion of varicella–herpes zoster virus in breast milk (Frederick et al). 1986; **154**:1116-7
Herpes simplex virus testing of an obstetric population with an antigen enzyme-linked immunosorbent assay (Warford et al). 1986; **154**:21-8
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15

Vitamin A
The effect of antenatal dexamethasone on maternal and fetal retinol-binding protein (Hustead and Zachman). 1986; **154**:203-5

Vitamin E
Influence of breast-feeding on the restoration of the low serum concentration of vitamin E and β-carotene in the newborn infant (Ostrea Jr et al). 1986; **154**:1014-7

Vulva
Focal vulvitis: A characteristic syndrome and cause of dyspareunia: Features, natural history, and management (Peckham et al). 1986; **154**:855-64
The role of lymphangiography in vulvar carcinoma (Weiner et al). 1986; **154**:1073-5

W

Wart virus
Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection (McArdle and Muller). 1986; **154**:509-15

Water-soluble contrast material
Disagreement on use of water-soluble contrast material in small bowel obstruction (Ott et al) (Letter); (Robertson) (Reply). 1986; **154**:690-1

Weight; *see also* **Birth weight**

Weight
Effect of weight loss and antiandrogenic therapy on sex hormone blood levels and insulin resistance in obese patients with polycystic ovaries (Pasquali et al). 1986; **154**:139-44

Prepregnancy weight, weight gain, and birth weight (Abrams and Laros Jr). 1986; **154**:503-9

Y

Y chromosome
Use of two different deoxyribonucleic acid probes to detect Y chromosome deoxyribonucleic acid in subjects with normal and altered Y chromosomes (McDonough et al). 1986; **154**:737-48

Z

Zona-free hamster egg penetration test
An evaluation of different media for the zona-free hamster egg penetration test (Mortimer). 1986; **154**:351-4

Zuclomiphene citrate
The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo (Schmidt et al). 1986; **154**:727-36

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Professor and Head
Section on Maternal-Fetal Medicine
or
Frank C. Greiss, Jr., M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
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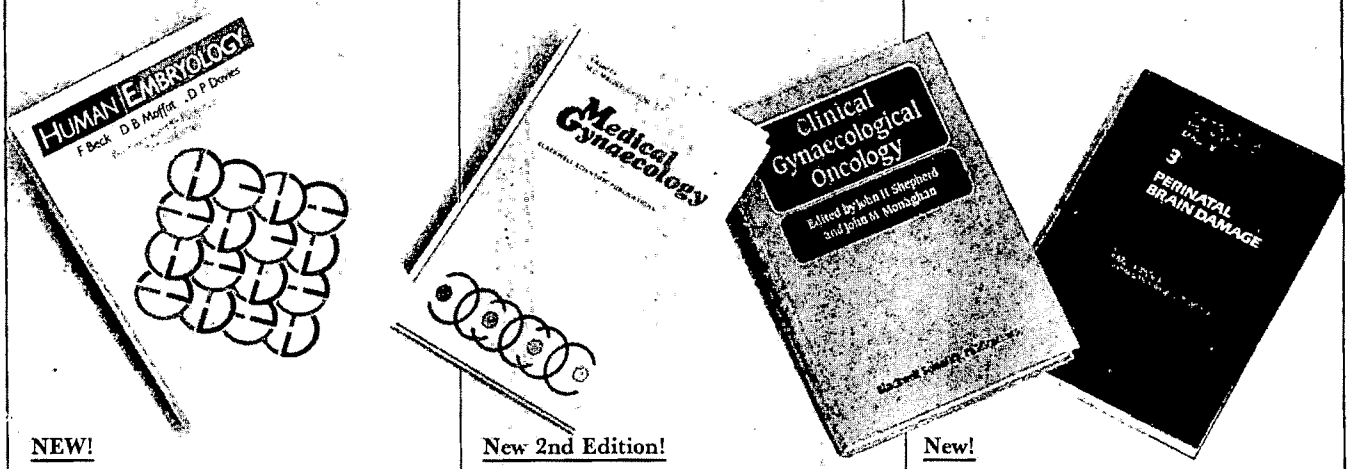
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INDEX TO ADVERTISERS

Abbott Pharmaceuticals, Inc.
Ogen 17A,18A,19A

Accurate Surgical & Scientific Instruments Corporation
Surgicraft Copeland 12A

Baylor College of Medicine
Opportunity Available 45A

Berlex Laboratories, Inc.
Tri-Levlen 42A,43A,44A

Bowman Gray School of Medicine
Opportunity Available 45A

College of Physicians & Surgeons/Columbia University
Annual Review Course 41A

Dean Medical Center
Opportunity Available 46A

FHP Professional Staffing
Opportunity Available 47A

Faculty of Medicine of Columbia University
Opportunity Available 46A

Georgetown University School of Medicine
Opportunity Available 41A

Group Health Plan
Opportunity Available 47A

Kaiser Permanente Medical Center
Opportunity Available 36A

Lutheran General Hospital
Opportunity Available 48A

Marshfield Clinic
Opportunity Available 46A,48A

Mead Johnson Laboratories
Estrace 28A,29A,30A

Medical Center of Georgia
Opportunity Available 48A

Medical Equipment Designs, Inc.
HemoCue 14A,15A

Merck Sharp & Dohme
Mefoxin 34A,35A,36A

Miles Pharmaceuticals
Mycelex-G 8A,9A,10A

Mosby Company, The C. V.
Books 51A

Nassau County Medical Center
Opportunity Available 47A

National Institutes of Health
Opportunity Available 45A

NYU Medical Center Post-Graduate Medical School
Course 32A

Ortho Pharmaceutical Corporation
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cover

Patuxent Medical Group
Opportunity Available 47A

Purdue Frederick
Betadine 6A

Saint John Hospital
Opportunity Available 49A

Smith Kline & Beckman
Cefizox 20A,21A

Southern Colorado Clinic
Opportunity Available 46A

Southern Illinois University School of Medicine
Opportunity Available 49A

Springer-Verlag
Books 23A

Stanford University School of Medicine
Opportunity Available 50A

Syntex
Femstat 37A,38A,39A,40A
Tri-Norinyl ..... Second Cover, 1A,2A

UCLA School of Medicine
Opportunity Available 50A

UMDNJ-New Jersey Medical School
Opportunity Available 49A

Upjohn
Provera 3A,4A

University of Michigan Medical Center
Opportunity Available 36A,49A

VHA Physician Placement Services
Opportunity Available 50A

VLI Corporation
Today Sponge 33A

Yale University Health Services
Opportunity Available 50A

Year Book Medical Publishers
Books 31A

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INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen.

CONTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may cause fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who are pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study conducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than that of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study conducted in the United States found that past use of oral contraceptives was associated with increased risk of subarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk may be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists following discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the past use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is discontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY. BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert).** **DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 12/84

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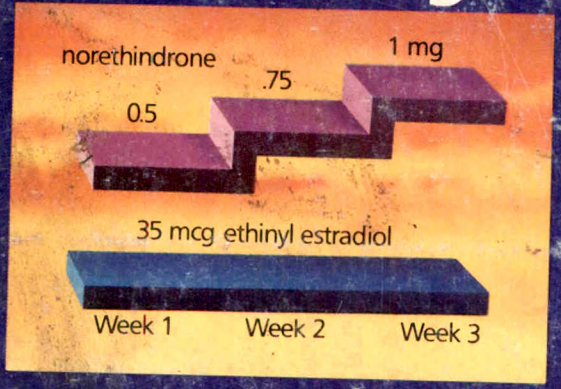
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